VU Research Portal

2-Alkyl-1-(ortho-alkyl-phenyl) cyclohexanols

Timmerman, H.

1967

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
Timmerman, H. (1967). *2-Alkyl-1-(ortho-alkyl-phenyl) cyclohexanols: synthesis, conformation and some pharmacological investigations*.

2-ALKYL-1-[ORTHO-ALKYL-PHENYL]CYCLOHEXANOLS

SYNTHESIS, CONFORMATION AND SOME PHARMACOLOGICAL INVESTIGATIONS

H. TIMMERMAN

1620147

2-ALKYL-1-[ORTHO-ALKYLPHENYL]CYCLOHEXANOLS

SYNTHESIS, CONFORMATION AND SOME PHARMACOLOGICAL INVESTIGATIONS
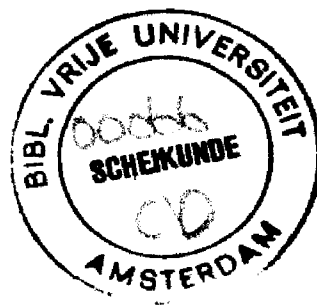
VRIJE UNIVERSITEIT TE AMSTERDAM

2-ALKYL-1-[ORTHO-ALKYL-PHENYL]CYCLOHEXANOLS

SYNTHESIS, CONFORMATION
AND SOME
PHARMACOLOGICAL INVESTIGATIONS

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van Doctor in de Wiskunde en Natuur-
wetenschappen aan de Vrije Universiteit te Amsterdam, op gezag
van de Rector Magnificus Mr. W. F. de Gaay Fortman, Hoogleraar
in de faculteit der Rechtsgeleerdheid, in het openbaar te verdedigen
op vrijdag 19 mei 1967 te 13.30 uur in het Woestduincentrum,
Woestduinstraat 16 te Amsterdam

door HENDRIK TIMMERMAN

geboren te Schoonebeek

PROMOTOR: PROF. DR. W. TH. NAUTA

Aan de nagedachtenis van mijn vader

voor mijn moeder

voor Anne-Wytske

ACKNOWLEDGEMENT

CONTENTS

PART I CHEMICAL INVESTIGATIONS

CHAPTER 1 INTRODUCTION

In the past decennia the conformation of cyclohexane and of its derivatives has been the subject of frequent investigation. When Hassel (1943,[53]) conceived the notions of axial and equatorial, the non-planar structure of the cyclohexane ring had since long been common knowledge. The recognition of these two possibilities for a substituent to be oriented opened the way to an explanation of a great many stability differences between isomers. The equatorial position of a substituent, as compared to its axial orientation, has come to be generally acknowledged as the more favoured.
Modern techniques, as the various spectroscopic methods, have considerably simplified conformation analysis.

Addition to a double bond of a carbonyl function, which is situated in an asymmetric molecule may lead to several isomeric compounds. With the aid of the rule of Cram it is possible to forecast how such an addition proceeds stereochemically [30, 31]. According to the rule of Cram, addition will take place from the least hindered side of the carbonyl group. In carbonyl compounds the C=O group is situated exactly between the smallest and the middle group (see fig. 1).
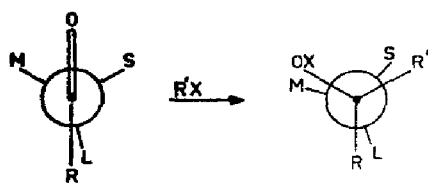
fig. 1 The rule of Cram.

L: greatest group
S: smallest group
M: middle group
R: substituent already present before addition
R' and X: group introduced by addition

In 2-alkylcyclohexanone the starting conformation appears as pictured below (fig. 2)[26]. The O-R angle is about 15'. Additions to cyclic compounds, however, even when the conditions of the rule of Cram are not met (a carbonyl function situated exactly between the smallest and the middle group, in an asymmetric molecule) nevertheless do show a selective effect, so that this rule evidently cannot be applied to cyclic ketones.
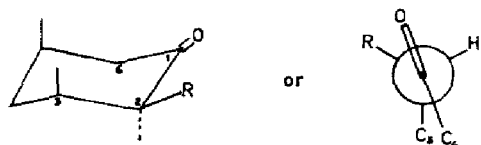
fig. 2   The conformation of 2-alkylcyclohexanones

The rule of Cram would further imply that both reduction with complex hydrides and reaction with a Grignard reagent will have a steric effect in the same direction. Actually, however, reactions show opposite steric selectivities.

In the case of reduction of cyclic ketones with complex metal hydrides we have the well-known theory of Dauben (1956[32, 33]), in which the author bases himself on a conformation of 2-alkylcyclohexanones identical to the one pictured in fig. 2. Beckett, when explaining the selectivity seen in the reduction of tropinon with complex metal hydrides, arrived at a similar theory[4].

In an earlier publication[95], we suggested the use of Dauben's theory to explain the selectivity in the reaction of cyclohexanones, which happens to be the reverse of that in the above mentioned reduction method. In their recent book, Eliel, Allinger, Angyal and Morrison suppose that for this kind of reactions the isomer ratio may indeed be explained with Dauben's theory[41]. In the present investigation the conformation was determined of the cyclohexanols, obtained by the reaction of ortho-substituted phenyllithium compounds with 2-alkylcyclohexanones, and the effect was studied of the ortho substituents, and of the 2 alkyl groups in the cyclohexane component. The conformation of the cyclohexanols was determined by means of NMR spectroscopy. The choice of this method was dictated by the fact that in all our cases only one isomer was obtained. All methods of conformation analysis are based on the possibility to *distinguish two isomers*. In NMR spectroscopy the difference between

12

an axial and an equatorial hydroxyl group is sufficiently large: the axially situated hydroxyl proton resonates at a higher field than an equatorial one.[79] There are furthermore good data, both from 1- as from 2-phenylcyclohexanols[48, 17] known in the literature, which can be used for comparison. A determination of the conformations could therefore be carried out with the aid of these data.

In the course of the investigation it proved necessary to synthesize also a number of carbinols related to α-trans-decalon. Their conformation was equally established with the aid of NMR spectroscopy.

1-phenylcyclohexanols may be considered as α-di-substituted benzyl-alcohols, and a series of ortho-alkylbenzylalcohols was therefore also included in the present investigation.

For the synthesis of all these, known literature methods were used.

2.1 ORTHO-ALKYLBROMOBENZENES

The 2-alkyl-1-(*ortho*-alkylphenyl)cyclohexanols were synthesized as described by Carlin[18], whereby 1 aeq. of a cyclohexanone solution in ether was added slowly to 1 aeq. of aryl-lithium in ether, obtained from arylbromide and Li.

The following brominated compounds were used:

ortho-methyl-, *o*-ethyl-, *o*-n-propyl-, *o*-n-butyl-, *o*-i-propyl-, *o*-tert.-butyl-, *o*-tert.amyl-, 2,6-dimethyl- and 2,6-diethylbromobenzene.

The 2,6-dimethyl and the 2,6-diethyl compounds were available from the laboratory stocks.

Ortho-methyl-, *o*-ethyl-, *o*-n-propyl-, *o*-n-butyl- and *o*-isopropyl-bromobenzene were obtained from the corresponding anilines via the diazo-compounds, in a manner as described for *o*-bromotoluene in Organic Synthesis[6].

The results and some physical data of the o-alkylbromobenzenes have been listed in table 1.

TABLE 1

Some physical constants of *o*-alkyl-bromobenzenes

alkyl group	B.P.	N_D^{20}	N_D^{20}lit.	yield from aniline
CH_3	60-61.5/14	1.5561	1.5537[60]	42 %
C_2H_5	80-82/15	1.5486	1.5487[20]	40 %
n–C_3H_7	92/13	1.5394	1.5395[69]	40 %
n–C_4H_9	117-119/15	1.5292	1.5328[69]	40 %
i–C_3H_7	100.5/23	1.5395	1.5412[69]	37 %

Ortho-methyl-, *o*-ethyl- and *o*-isopropylaniline [a]) were available from the laboratory stocks. *Ortho*-n-propyl and *ortho*-n-butyl-

aniline were prepared from the corresponding nitro-compounds by reduction with iron and hydrochloric acid.[71]

Ortho-nitro-n-butylbenzene and the corresponding n-propyl compounds were obtained in a way as depicted in reaction scheme 1, while table 2 shows the respective yields.
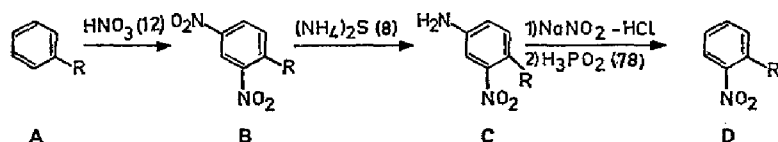
Reaction Scheme 1

TABLE 2

Yields of the reaction steps in reaction scheme 1 and some physical constants of D

R	$n-C_3H_7$	$n-C_4H_9$
yield B from A	83 %	57 %
C from B	39% (as hydrochloride)	33% (as hydrochloride)
D from C	55 %	44 %
B.P. D	109-110/9	123-124/12
N_D^{20} D	1.5285	1.5227

As the route to these relatively simple products seems rather long, we also nitrated n-butylbenzene according to Read and Mullin, in spite of the statement by Crawford and Stewart that the reaction mixture obtained by mono nitration of n-alkylbenzenes contains only a slight amount of *o*-alkyl-nitrobenzenes.[84, 29] The obtained mixture was separated in its components by distillation over a heligrid column with a capacity of 15 theoretical plates.

Identification was possible by means of the IR spectra, those of *o*- and *p*-nitroalkyl benzenes showing one characteristic difference: the p-derivatives show a band at 12 μ which is lacking in the case of the corresponding *o*-compounds.[81] A fraction was obtained (yield: 30 %), boiling range 133-139°/15 and N_d^{20}: 1.5232, which

15

proved identical with the product obtained in the first described manner.

The *para*-isomer was also obtained in a 30 % yield; boiling point 150-152°/17, N_D: 1.5305. Its IR spectrum indeed revealed a band at 11.87 μ, which was also found in the intermediate fraction, but not however, in the first fraction.

The two tert.alkyl compounds were synthesized according to Shoesmith and Mackie.[88] In the bromination step use was made, however, of Ag_2SO_4, in equimolecular amounts in concentrated H_2SO_4, according to the method of Derbyshire and Waters[34]. The required tert.pentylbenzene was obtained according to Huston, from benzene and tert.pentanol.[61]

The physical constants of both compounds are listed in table 3.

TABLE 3

Physical data of o-tert.alkyl bromobenzenes

tert. alkyl group	overall yield from hydrocarbon	B.P.	N_D^{20}
$t-C_4H_9$	40 %	94.5-95.5/12	1.5442
$t-C_5H_{11}$	16 %	106-108/11	1.5372

2.2 2-ALKYLCYCLOHEXANONES

Most of the 2-alkylcyclohexanones are commercial products, but gaschromatographic analysis indicated the presence of considerable amounts of impurities. For the purpose of purification we therefore prepared the semicarbazones according to the method of Carlin, which makes use of a buffered solution.[19] The only exception was o-tert.butylcyclohexanone, in which case the yield is too low.

The cyclohexanones are recovered by hydrolysis in diluted H_2SO_4. In addition, 2-isopropylcyclonexanone was synthesized by hydrogenation of o-isopropylphenol and subsequent oxydation of the cyclohexanol:

500 g of o-isopropylphenol, 200 ml of alcohol 96 % and 15 g of RaNi were fed into an autoclave. Several pellets of NaOH were added [7]). Hydrogen absorption started at 90 atm. H_2 and 70°. Additional hydrogen was introduced repeatedly until it was no longer perceivably absorbed.

The temperature was then increased to 150°; when at this temperature no longer hydrogen was absorbed, the reaction mixture was allowed to cool. After removing of the catalyst the mixture obtained yielded crude 2-isopropylcyclo-hexanol.

This was oxydized according to the method of Brown [15]), with the aid of sodium bichromate, in a vigorously stirred ether-water mixture.

The cyclohexanone yield was over 90 %, based on the phenol.

2-Tert.butylcyclohexanone was obtained either starting from 2-tert.-cyclohexylacetate [a]), by hydrolysis and oxydation, or from the corresponding phenol in a similar way as the isopropyl derivative. In this case the circumstances for the hydrogenation were as follows: the absorption of hydrogen started at 120° and 120 atm. H_2. The reaction then proceeded in the same way as described above. Since the purification of 2-tert.butylcyclohexanone could not be carried out via the semicarbazone, it was distilled over a spinning band column.

After this effective distillation, only $\pm$ 0.1 % of impurity could be demonstrated by GLC analysis.

The physical data of the 2-alkylcyclohexanones and of their respective semicarbazones are listed in table 4, together with the values reported in the literature.

Some semicarbazones distinguished themselves by displaying a varying melting point which also deviated from the literature data. The discrepancy was particularly marked in the case of the 3-n-propylcyclohexanone semicarbazone; in a few of the other compounds recrystallisation resulted in a lowering of the melting point of about 15°. It is definitely impossible to explain this phenomenon by the presence of small amounts of solvent.

Elementary analysis carried out in the case of some of the products with the largest discrepancies yielded satisfactory results, and GLC analysis showed the ketones recovered from these semicarbazones to be single products.

That the literature values also differ strongly between one another, has already been pointed out by Radell, also in the case of the aliphatic ketones.[83]

———————

[a]) The author is indebted to Dow Chemical International Inc. Brussels, Belgium (now established at Rotterdam, The Netherlands) for supplying samples of 2-isopropylcyclohexanone and 2-tert.butylcyclohexylacetate.

17

TABLE 4

Some physical data on 2-alkylcyclohexanones

2-alkyl group	source	M.P. semi-carbazone	the same in literature	B.P.	N_D^{20}	the same in literature
CH$_3$	lab. stock	not constant 1: 190-193 2: after re-crystal. 178-181 a)	191 [19,89]	53/12	1.4487	1.4488 [89]
C$_2$H$_5$	lab. stock	162-163	161, 165 [35] 166 [94]	60.5-61.5/12	1.4521	1.4530 [16°] [94]
n-C$_3$H$_7$	lab. stock	116.5-133 b) c)	119-120 [35] 130 [94] 134-135 [75]	70-70.5/8	1.4532	—
n-C$_4$H$_9$	lab. stock	146.5-148	150 [94]	93.5-95/11	1.4554	1.4522 [25°]
i-C$_3$H$_7$	lab. stock	not constant 1: 199-200 2: after re-crystal. 174-177	187 [75] 180 [94]	81/17	1.4552	1.4564 [15°]
i-C$_3$H$_7$	ortho-iso-propyl-phenol	183.5-184	—	73.5-74/10	1.4559	—
t-C$_4$H$_9$	2-tert. butyl cyclo-hexylacetate d)	—	—	91.5-92/22	1.4582	1.4570 [25°] [49]
t-C$_4$H$_9$	ortho-tert. butylphenol	—	—	89-90/17	1.4579	—

a) C$_8$H$_{15}$N$_3$O (169.2): C 56.72, H 8.93, N 24.83
found: C 56.8 , H 8.8 , N 24.7
b) C$_{10}$H$_{19}$N$_3$O (197.3): C 60.87, H 9.70, N 21.25
found: C 60.9 , H 9.7 , N 21.6
c) The compound melts in two parts: the first at 116.5, the second at 131-133.
d) See note on page 17.

A possible explanation for this might be that a syn-anti-isomerism is involved, with the isomers present in varying ratio. The fact that the semicarbazone of 2-n-propylcyclohexanone does distinctly melt in two separate stages, would seem an argument in favour of this theory, although the semicarbazones of some symmetric ketones do equally show a melting point which is far from constant.[83]

18

2.3 2-ALKYL-1-(ORTHO-ALKYLPHENYL)CYCLOHEXANOLS

By dropwise addition of a 2-alkylcyclohexanone solution in ether to an aryl-lithium compound about twenty-five hexanols were obtained in all.

The general procedure was as follows:

About 25 ml of anhydrous ether were placed in a 250 ml three-necked flask, through which nitrogen had been passed for ± 45 minutes.

Under a steady nitrogen-flow 0.2 moles of lithium (small clippings of freshly pressed wire) were added, followed by a solution of 0.1 mole of o-alkylbromo-benzene in 150 ml of dry ether added dropwise. The reaction started quickly and the mixture assumed a yellowish colour.

After addition was complete the mixture was boiled for four hours.

While cooling in an ice bath, a solution of 0.1 mole of 2-alkylcyclohexanone in ± 25 ml of ether were added dropwise, after which the mixture was boiled for another 3 hours. The yellow colour persisted. The mixture was left overnight under nitrogen and then poured into water.

The organic layer was separated, the aqueous layer extracted with ether a few times, the ethereal solution washed until neutral, dried and distilled from a Claisen flask. The compounds cannot be distilled through a column with a metal filling, because this may result in almost 100 % dehydration; however, small amounts of dehydrated products can hardly be avoided.

TABLE 5

Some data on 2-alkyl-1-(ortho-tert.alkylphenyl)cyclohexanols
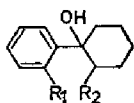
com- pound	R_1	R_2	boiling point	N.M.R. δOH 0.75 mol CCl$_4$	N_D^{20}	elementary analysis				yield from the cyclo- hexanone
						$C_{calc.}$	C_{found}	$H_{calc.}$	H_{found}	
XVIII	t-C$_4$H$_9$	H	109-113/10^{-2}	1.75	Solid m.p. 53-56	82.76	82.7	10.35	10.4	60 %
XIX	t-C$_4$H$_9$	CH$_3$	110-112/10^{-2}	1.77	1.5330	82.92	82.3	10.53	10.1	38 —
XX	t-C$_4$H$_9$	C$_2$H$_5$	132.5-134/10^{-2}	1.77	1.5340	83.08	82.4	10.77	10.6	20 —
XXI	t-C$_4$H$_9$	n-C$_3$H$_7$	126-129/10^{-2}	1.75	1.5245	83.21	83.7	10.95	10.8	30 —
XXII	t-C$_4$H$_9$	n-C$_4$H$_9$	128-130/10^{-2}	1.74	1.5234	83.33	83.1	11.11	11.0	30 —
XXIII	t-C$_5$H$_{11}$	C$_2$H$_5$	119-125/10^{-1}	1.72	1.5286	83.21	83.5	10.95	11.2	35 —

The yields vary widely; they depend of the substituents present. In particular the presence of a tert.butyl group, whether in the phenyl group or in the saturated nucleus, may be held responsible for strongly diminished yields.

Data of compounds containing an ortho-tert.butylphenyl group are reported in table 5, those of the other compounds synthesized may be found in table 6.

TABLE 6

Some data on 2-alkyl-1-arylcyclohexanols
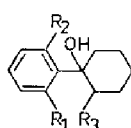
com-pound	R_1	R_2	R_3	boiling point	N.M.R. δ OH 0.75 mol CCl₄	N_D^{20}	elementary analysis				yield from the cyclo-hexanone
							$C_{calc.}$	C_{found}	$H_{calc.}$	H_{found}	
I	H	H	t-C_4H_9	120-124/1	1.46	1.5343	82.76	83.1	10.35	10.4	65 %
II	CH_3	H	CH_3	121-122/1	1.37	1.5426	82.35	82.3	9.80	9.7	84 — a)
III	CH_3	H	C_2H_5	124-125/2	1.40	1.5395	82.57	82.7	10.09	10.4	66 —
IV	CH_3	H	i-C_3H_7	142-144/2	1.48	1.5349	82.76	82.9	10.35	10.4	68 —
V	CH_3	H	t-C_4H_9	111-115/0.5	1.49	1.5334	82.92	82.3	10.53	10.3	22 —
VI	C_2H_5	H	CH_3	129-133/3	1.43	1.5383	82.57	82.0	10.09	10.1	83 —
VII	C_2H_5	H	C_2H_5	108-111/10⁻¹	1.40	1.5360	82.76	82.7	10.35	10.4	70 —
VIII	C_2H_5	H	i-C_3H_7	134-136/2	1.40	1.5329	82.92	82.8	10.53	10.7	65 —
IX	C_2H_5	H	t-C_4H_9	130-135/2	1.49	1.5348	83.08	82.9	10.77	10.7	25 —
X	i-C_3H_7	H	CH_3	96-100/10⁻²	1.43	1.5343	82.76	82.7	10.35	10.5	81.5 —
XI	i-C_3H_7	H	C_2H_5	99-100.5/10⁻²	1.41	1.5319	82.92	82.6	10.53	10.6	75 —
XII	i-C_3H_7	H	i-C_3H_7	127.5-129/0,5	1.45	1.5279	83.08	82.9	10.77	10.6	45 —
XIII	i-C_3H_7	H	t-C_4H_9	138-142/10⁻¹	1.46	1.5288	83.21	82.5	10.95	10.8	11 —
XIV	n-C_3H_7	H	n-C_3H_7	133-134/0,5	1.45	1.5208	83.08	82.6	10.77	10.6	37 —
XV	n-C_4H_9	H	n-C_4H_9	142/10⁻²	1.25	1.5136	83.33	82.6	11.11	11.1	35 —
XVI	CH_3	CH_3	C_2H_5	110-122/0,5	1.46	1.5393	82.76	82.6	10.35	10.5	50 —
XVII	C_2H_5	C_2H_5	C_2H_5	116/10⁻³	1.45	1.5251	83.08	82.5	10.77	10.8	12 —

a) Carlin (19), B.P. 117/1, N_D^{25} 1.5377, 82 %.

CHAPTER 3   STEREOCHEMICAL ASPECTS OF THE REAC-
TION BETWEEN 2-ALKYL-CYCLOHEXANONE
AND AN ARYLLITHIUM COMPOUND; THE
CONFIGURATION OF THE PRODUCTS OB-
TAINED

3.1 THE THEORY OF DAUBEN

The theory of Dauben et al. on the stereochemical course of the reduction of cyclic ketones with complex metal hydrides is based on the assumption that the hydride ion is able to attack the carbonyl group from two sides.

If the ring is approached from above – axial approach –, the result is the formation of an axial H and an equatorial hydroxyl group. An approach from below the ring – equatorially – results in the opposite conformation.

Dauben states that if the original chances for both approaches are equal, the most stable conformer, which is created by axial approach and which therefore carries the relatively bulky hydroxyl group equatorially, will be formed in excess.

In a subsequent publication he elaborates on this effect, which he himself calls the "product development control", by stating that in the case of *pure* product development control the isomer relations in the reaction mixture will be determined by the energy levels of the transition states.[33] These being of course unknown, he contents to use as a measure the differences of the energy *contents* of the cyclohexanols to be found.

Although Dauben introduces an energy factor here, he does not neglect the steric influence, which so far had been considered the sole factor determining the selectivity in this kind of reactions. For it is definitely unjustified to grant both approach possibilities an equal chance. The upper approach will be hindered considerably by the substituents in positions 3 and 5, even when only hydrogen atoms are present. This will therefore constitute a "steric approach control", which favours the equatorial approach and hence the creation of the less stable isomer.

Literature data seems to confirm the theory of Dauben: in particular the work of Eliel et al., who made use of $LiAlH_4$-$AlCl_3$ mixtures

21

demonstrates its usefulness.[65, 39] However, the product development control is an effect which is hard to formulate accurately. If the reactions conditions do not allow equilibrium to be attained, the amounts of both isomers will be governed solely by the rate at which they are created, hence by the respective activation energies. The formation, in a certain reaction, of a greater amount of the stable isomer than would be present in an equilibrium, is caused by the fact that the activation energy of the reaction which leads to the stable isomer is relatively low.

If we therefore assume that in the reduction of cyclic ketones with for instance $LiAlH_4$, both approaches have an equal chance, the difference of the activation energies of the reactions will determine the relative amounts of the isomers formed. The results found by Dauben would indicate the activation energy of the "axial reaction" as the lower one.

3.2 STEREO SELECTIVITY IN THE REACTION BETWEEN A CYCLOHEXANONE AND AN ORGANOMETALLIC REAGENT

The reaction of the cyclohexanones with compounds of the Grignard type and Li-compounds shows selectivity as well. Recent literature has also endeavoured to explain this selectivity with the above expounded theory.[40, 57, 73]

Over and again it is assumed that increased hindrance will cause formation of an excess of the product with the hydroxyl group in axial position. Equatorial approach affords a product with the substituent in equatorial, and the hydroxyl group in axial position.

The larger the bulk of the introduced group, the stronger is the "steric approach control". The resulting product will now be relatively stable. In this case, axial approach is considerably more hindered than in the reduction reaction, and will moreover afford the less stable isomer; that is, if the OM group is smaller than the new substituent, which is certainly true in the case of an aryllithium addition. The possibility of an opposed factor, as conceived by Dauben, can be disregarded with this kind of reactions.

Table 7 provides an incomplete summary of the literature data concerning the selectivity of these reactions.

There is no doubt about the equatorial approach being favoured.

TABLE 7

Selectivity in reactions between cyclic ketones and organo-metallic reagents
A literature survey

ketone	Grignard reagent	% isomer from equat. approach	lit.
2-methylcyclohexanone	CH_3MgI	75	[77]
3-methylcyclohexanone	CH_3MgI	60	[64]
4-tert.butylcyclohexanone	CH_3MgBr	55-60	[56]
3-tert.butylcyclohexanone	C_2H_5MgBr	± 85	[82]
4-tert.butylcyclohexanone	C_2H_5MgBr	73	[56]
4-tert.butylcyclohexanone	C_6H_5MgBr	± 50	[48]
2-CH_2OCH_3-cyclohexanone	C_6H_5MgBr	100	[103]
2-CH_2OCH_3-cyclohexanone	C_6H_5Li	100	[103]
1.3.5-tri-methyl-4-piperidone	C_6H_5Li	95 [a]	[92]
Tropinon	C_6H_5Li	97	[3]
1.3-di-methyl-4-piperidone	o-$CH_3C_6H_4Li$	100	[3]
1.3-di-methyl-4-piperidone	m-$CH_3C_6H_4Li$	90	[3]
1.3-di-methyl-4-piperidone	p-$CH_3C_6H_4Li$	> 50	[3]
1.3-di-methyl-4-piperidone	o-$OCH_3C_6H_4Li$	100	[3]
some N-substituted 3.5-di-methyl-4-piperidone	some aryl-lithium compounds	100	[52]
2-piperidone-cyclohexanone	some alkyl and aryl Grignards	100	[38]

[a]) Sorokin did obtain the 5 % yield of compound with an equatorial hydroxyl group, only when using the hydrochloride of the ketone. He does not report any spectral data and it is therefore probable that what one has here, is a boat form, rather than the structure as suggested by Sorokin.

The above table lists results of the last decennium only; in earlier work, such as there is, usually only a single isomer is mentioned, where present methods might well be able to distinguish two isomers formed.

The survey shows that the composition of the reaction mixture is definitely highly dependent of the steric hindrance round the carbonyl function, as well as of the size of the group round the metal atom in the organo metallic reagent.

Generalizing one may say that the selectivity of these reactions is in a large measure determined by steric factors.

This is very clearly illustrated by the results of Becket et al. who were able to demonstrate but one isomer in the case of an addition of ortho substituted phenyllithium compounds to 1,3-di-methyl-4-piperidone, while when meta or para-substituted compounds were used, two isomers were formed, one of them in large excess.[3] The configurations have been established by comparison of the obtained isomers with compounds of the prodine type [a]) of known configuration. The product obtained in the case of ortho substituted phenyl compounds has a similar configuration as that of the main product of the other reactions.

The table furthermore shows that substitution on the 2-position of the cyclic ketone also favours the creation of the compound with the hydroxyl group in axial position.

This is in agreement with the theory that steric factors determine the selectivity of this type of reactions.

3.3 PROBABLE CONFIGURATION OF THE SYNTHETISIZED CYCLOHEXANOLS

The syntheses which we carried out, and in which there were always one or more substituents present in the ortho-positions of the benzene ring and/or in the 2-position of the cyclohexanone, did invariably yield but one of the two possible isomers. This was certified with the aid of gas-liquid chromatography using columns with various polar and non-polar compounds as filling material. Nearly always there was a second fraction, but this is probably formed during the chromatographical procedures. If the main fraction is collected and repeatedly subjected to chromatography, the same fraction occurs again. This is very likely due to dehydratation, a symptom also witnessed when distillations over a heligrid column were carried out; in some cases this even causes complete dehydratation. According to the NMR spectrum the product obtained contains the 1- and the 6-cyclohexene compound in roughly the same amount. We may conclude from the above that the products obtained are all formed by equatorial approach of the carbonyl function. However, the

---

[a]) Prodine is the hydrochloric salt of the propionyloxy ester of 1,3-dimethyl-4-phenylpiperidine-4-ol.

24

ultimate form of the resulting configuration is directly related to the conformation of the ketone.

It has been quite a long while ago since it was suggested that the conformation preferences of the 2- and 3-alkyl groups in the cyclohexanones would be unlike those of the corresponding groups in the cyclohexyl compounds. The phenomenon was referred to as the "2- and 3-alkylketone effect".[67, 87]

Any such effect could account for the lesser stabilisation of alkyl groups in equatorial position in 2- and 3-alkylcyclohexanones as compared to the situation in the corresponding cyclohexanes. In the case of 2-alkylcyclohexanone the effect is brought about by repulsion between the carbonyl and the alkyl group.

Recent investigations revealed that a 2-alkyl ketone effect does definitely not occur in the case of 1-methylcyclohexanone. Although in the cases of 2-ethyl- and 2-i-propylcyclohexanone the energy advantage of an equatorial is considerably less, the equatorial position remains nonetheless energetically more favourable.

TABLE 8

Conformation and thermodynamic data on alkylcyclohexanes and 2-alkylcyclohexanones
A literature survey

alkyl group	alkylcyclohexane		2-alkylcyclohexanone				cyclohexanone used as model
	$\triangle G_{e \to a}$[a] Kcal/mol.	lit.	$\triangle H_{e \to a}$ Kcal/mol.	$\triangle S_{e \to a}$ e.u.	temp.	lit.	
methyl	1.5–2.1	[41]	2.16	$+0.8 \pm 0.2$	78.5	[27]	4-tert.butyl-2-methyl-
	1.7		1.57	$+0.1 \pm 0.6$	64.0	[1]	4-tert.butyl-2-methyl-
ethyl	1.65–2.25		1.26	-0.8	78	[28]	2.6-di-ethyl-
	1.8	[41]	1.28	$+0.2$	—	[86]	2.6-di-ethyl-
			1.6	0	64.6	[1]	4-tert.butyl-2-ethyl-
iso-propyl	1.8–2.5	[41]	0.32	-0.8	—	[86]	2.6-di-isopropyl-
	2.1		0.44	-0.5 ± 0.3	64	[1]	4-tert.butyl-2-isopropyl-
tert. butyl	5.6	[41]	2.39 b)	$+2.5$	80.3	[1]	2.4-di-tert.butyl-

a) The authors (16) assumed that S = 0, for various temperatures.
b) Instead of e → a (equatorial → axial),
   here read cis → trans.

25

In table 8 a summary is given of the literature data on different thermodynamic parameters of the alkyl group in alkylcyclohexanes and in 2-alkylcyclohexanones.

In the case of the methyl and ethyl group it can be deduced that these groups if substituted in the 2-position are for the overwhelming part present as equatorial substituents.

The values found by Allinger for the tert.butyl group indicate that 2-tert.butylcyclohexanone is largely present in boat form, which allows the author to explain the considerable entropy effect.[1]

The 2-isopropylcyclohexanone is mainly present in the conformation with the iso-propyl group in equatorial position.

In the given scheme the various possibilities for the reactions have been depicted.

For the 2-methyl and the 2-ethylcyclohexanones reaction a. (see scheme 2) is most likely and for the 2-tert.butylcyclohexanone in the boat form c. would be most favoured, in all cases for steric reasons.

For the same reasons as given above b. and d. are not probable.

Scheme 2
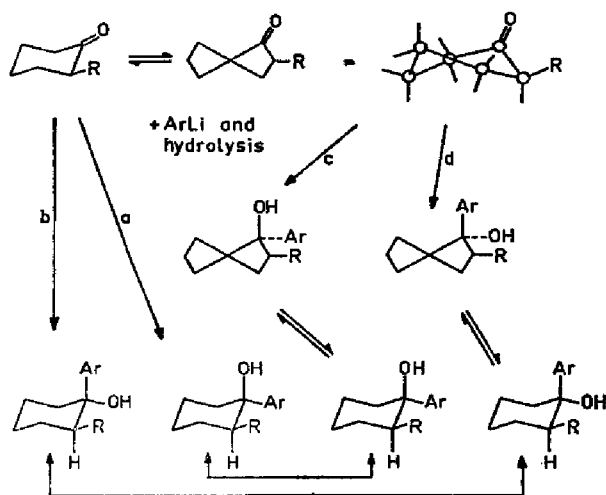
The 2-tert.butylcyclohexanone is for a great part present in the boat form. An approach from the side where R (the large tert.butyl group) is present is hindered. The introduced aryl group will therefore be situated trans, with regard to the tert.butyl group. When the formed cyclohexanol changes into the chair form, the final result of approach as depicted in c. will be identical to that according to b.

26

Hence the most probable conformation equilibria are the following:
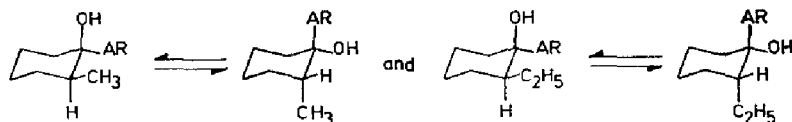
In the case of the 2-iso-propyl compounds, where in the ketone neither isomer is present in excess, a mixture might be expected.
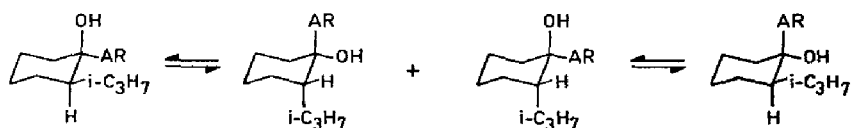
GLC analysis nevertheless revealed only a single isomer. In view of the above consideration we can not predict which isomer is obtained, but we consider a formation from the ketone with an equatorially oriented isopropyl group as most likely.

For the 2-tert.butyl-1-(ortho-alkylphenyl)cyclohexanol too, there is but one position possible (excluding the boat position); the conformation is moreover also determined as a result of the conformation homogeneity of the tert.butyl group.
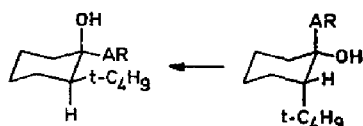
As reliable reference values for the protons of axially and equatorially oriented hydroxyl groups, both with a gem.phenyl group, as with a phenyl group on the 2-position of the cyclohexane ring, are available, it seems possible to determine the position of the equilibrium for the methyl and the ethyl derivatives, with the aid of NMR spectroscopy and the equation of Winstein and Holnes.[48,17,102] We are less certain in the case of the 2-isopropylcyclohexanols, while the 2-tert.butylcyclohexanols in the chair position show conformation homogeneity.

The Δ G values of the hydroxyl groups being substantially inferior to that of a phenyl group, viz. 0.7, respectively 3.1-3.2, it may be

reasonably expected that in the event of an equilibrium between the conformers, the said equilibrium will be mainly situated on the side of the conformers which carries the phenyl group equatorially.[48, 41] Moreover the Δ G value of the 2-alkyl group should be added to the existing difference between the Δ G values.

4.1 THE CONFORMATION AT C_1

When using NMR spectra to distinguish between the geometric isomers of cyclohexane compounds, we do usually rely on the fact that one of the protons in the ringsystem is separately recognizable.[17] Influenced by a geminal substituent, the signal of such a proton will have shifted to higher δ-values and will hence no longer coincide with the broad, complex, peak which is commonly caused by the protons of a cyclohexane system.

But in the case of 2-alkyl-1-arylcyclohexanol such a distinctive proton in the NMR spectrum is not available; neither can axial and equatorial positions of either phenyl or alkyl groups be clearly distinguished, so that only the hydroxyl group remains to base the conformation analysis on. The resonance of the proton of an axial hydroxyl group is found at higher field than the signal of its equatorially oriented equivalent.[79]

Carr differentiated between some 2-tolylcyclohexanols, – cis and trans –, by means of the NMR signal of both 1-, and the 2-proton, which resonate both at higher δ-values as the other ring-protons, and by means of the differences in the coupling constants for axial and equatorial protons, which permits a distinction to be made between the 1- and the 2-proton.[17] The values of the half widths agree with those of corresponding compounds, which are undoubtedly present in the chair form. The values reported by Carr, for the resonance of the hydroxyl protons (see table 9), may well serve as reference values.

The discrepancies between the results of Garbisch (see table 9) and Carr may be caused by the use of different solvents.

We determined the NMR spectra of all our compounds (Varian A60). Measurements were carried out at a constant temperature of 30° and all solutions were 0.75 molar in CCl_4. As an internal reference we used tetramethylsilane; the resonance values in table 5 and 6 are based on tetramethylsilane $\sim$ 0 ppm. The hydroxyl proton signals are easily recognizable by the small shift to higher field, which occurs when the sample is diluted.

TABLE 9

Chemical shifts of the hydroxyl proton in some 1-arylcyclohexanols

compound	conformation hydroxylgor up	δ OH-proton	solution	lit.
cis-2-o-tolyl-cyclohexanol	axial	1.40 p.p.m.	1 n CCl$_4$	[17]
trans-2-o-tolyl-cyclohexanol	equatorial	1.77 p.p.m.	1 n CCl$_4$	[17]
cis-2-m-tolyl-cyclohexanol	axial	1.34 p.p.m.	1 n CCl$_4$	[17]
cis-2-p-tolyl-cyclohexanol	axial	1.48 p.p.m.	1 n CCl$_4$	[17]
trans-4-t-butyl-1-phenylcyclohexanol	equatorial	1.91 p.p.m.	10 % CDCl$_3$	[48]
cis-4-t-butyl-1-phenylcyclohexanol	axial	1.65 p.p.m.	10 % CDCl$_3$	[48]

These shifts are usually very slight, and may be totally absent, as for instance in the case of the di-n.butyl derivative (XV. table 6) this may be due to the circumstance that association is severely hindered by the long carbon chains of the substituents. The relatively low values of both the NMR resonance signal of the hydroxyl proton, and of the refractive index, support this view.

For some compounds, (X-III), NMR spectra were determined for a series of concentrations. The results, together with the data reported by Ouellette for 4-tert.butylcyclohexanol – cis and trans – and for cyclohexanol, have been depicted in fig. 3.

The concentration dependency of the OH-shift of the 1-phenyl-cyclohexanols is clearly of a very minor character. In these compounds association will be inhibited by steric hindrance around the hydroxyl group. The intermolecular association will furthermore be hindered by possible internal association with π-electrons of the benzene ring.

The shifts of the compounds listed in table 6 agree with those reported by Carr for the axially oriented hydroxyl group. The values in table 5 correspond in a similar way with those of an equatorial hydroxyl group.

In a CDCl$_3$ solution, of the same concentration, the OH signal of II is shifted to 1.63 ppm, and that of XIX to 1.93 ppm. These data
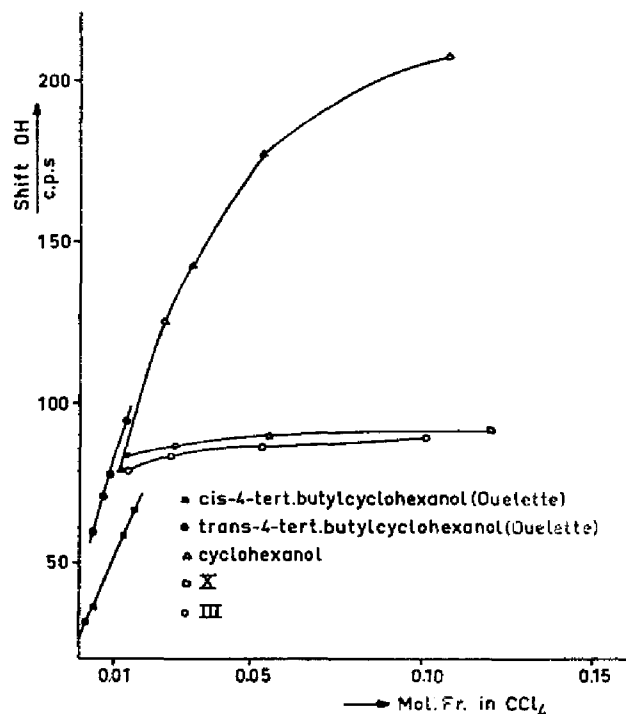
fig. 3 Relation between OH-shift and concentration of some carbinols in CCl_4.

agree with the values given by Garbisch for axial and equatorial hydroxyl groups.

The explanation given by Garbisch, according to which the difference in shift between the hydroxyl protons in cis- and trans-4-tert.butyl-1-phenylcyclohexanol, is caused by the difference in association, is but partly true.

In view of the minor concentration dependency of the shift, the relatively considerable differences observed between the isomers cannot be explained by differences in the degree of association. Being associated to a lesser degree only enhances the effect caused by the differing environment for respectively the axial and the equatorial position.

For the compounds II, VI and XIX we prepared the p-nitrobenzoyl esters (for the synthesis see page 42). The signal accorded to the hydroxyl group is no longer present in the NMR spectra of these esters.

If the shift of the resonance values of the hydroxyl protons of $\pm$ 1.45 ppm to $\pm$ 1.75 ppm had been gradual, a possible explanation could have been found in the fact that the ortho substituent by shielding the phenyl nucleus takes away some of its shielding effect

31

on the hydroxyl (the OH group may take up position in the deshielding zone of the benzene ring); this would cause the signal of the hydroxyl proton to shift to a higher ppm value, going from a primary to a tertiary ortho alkyl group in the benzene ring.[a])

However, as the transition occurs rather sudden, the phenomenon cannot be explained as in the above way.

A similar situation is found in the case of ortho-alkylbenzylalcohols; here too, an interaction is possible between the hydroxyl group and the π-electrons of the benzene ring. The 1-aryl-2-alkylcyclohexanols may be considered as α,α, disubstituted benzylalcohols.

If, for both groups of compounds the difference in shifts would indeed be based on a difference in shielding by the benzene ring, a similar effect should also be evident in the case of 2-alkylbenzyl-alcohols, with respectively primary, secundary and tertiary alkyl groups.

A series of ortho-alkylbenzylalcohols was therefore synthesized, starting from the ortho-alkyl-bromobenzenes and via the acid chlorides (see page 42). The physical constants are summarized in table 10.

Of these ortho-alkylbenzylalcohols NMR spectra were taken at different concentrations in CCl_4. The hydroxyl proton shift-concentration relation has been depicted in fig. 4.

The shift of the hydroxyl proton parallels clearly and in a very regular manner the increase in size of the ortho substituent of the benzylalcohols. Resonance occurs at higher field with increasing bulk of the ortho substituent (identical concentrations). Growing size of the ortho substituent will cause a decrease of the intramolecular associations, which in its turn will cause the resonance to occur at higher field.

The virtual absence of intermolecular association in the investigated cyclohexanols prevents this effect to show itself, when comparable concentrations are used.

There are indications in fig. 4 that all benzylalcohols included in the investigation display an identical shift at infinite dilution. The

---

[a]) The above is but a supposition. The shifts which we found, and the ones reported by Ouellette (fig. 3) for hydroxyl groups without geminal groups, all of them extrapolated towards infinite dilution, would seem to indicate that the hydroxyl proton is situated in the deshielding zone of the benzene ring.

TABLE 10

Some data on a series ortho-alkylbenzylalcohols

alkyl group	yield [a]	boiling point	the same in literature	N_D^{20}	the same in literature
H	from stock	89-90/10	93/10	1.5398	1.5395
CH_3	from stock	110-111.5/17	100-105/10	1.5420 [b]	1.5403 (25°)
$—CH_2—CH_3$	80%	117-120/16	—	1.5355	—
$—CH{\Large\langle}{}^{CH_3}_{CH_3}$	77 %	129.5-131/24	122-123/15	1.5270	1.5273
$—C{\Large\langle}{}^{CH_3}_{CH_3}{-}CH_3$	74 %	127-128/14	130-133/15	1.5327	—
$C{\Large\langle}{}^{CH_3}_{CH_3}{-}C_2H_5$ [c]	62 %	140-143/17	—	1.5261	—

[a]) from ortho-alkylbenzoylchloride.
[b]) this compound solidified after some time; M.P. 33-34°.
[c]) $C_{12}H_{18}O$ (178.27) C 80.85%, H 10.17%.
   found C 81.0 %, H 10.1 %.

strongest concentration dependency is found for the unsubstituted product.

The gradual transition has been depicted in table 11. There is a marked absence of any sudden change; in the case of the cyclohexanols such a change is caused by a change of the conformation at C_1.

On the basis of the available data the following conclusions may be drawn:

1 The cyclohexane ring in the 2-alkyl-1-arylcyclohexanols is present in the chair position; this follows from the agreement between our spectra and those reported by Carr, for some 2-tolyl-

TABLE 11

Relation ortho-substitution – chemical shift OH-proton in o-alkylbenzylalcohol and 1-(o-alkylphenyl)-2-methylcyclohexanol (both 0.5 mol. in CCl_4; c.p.s. relative to T.M.S.)
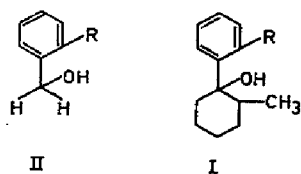
alkylgroup	shift OH in I	shift OH in II
H	81	189
CH_3	80	166
C_2H_5	84	161
i–C_3H_7	83	155
t–C_4H_9	108	150
t–C_5H_{11}	109	144
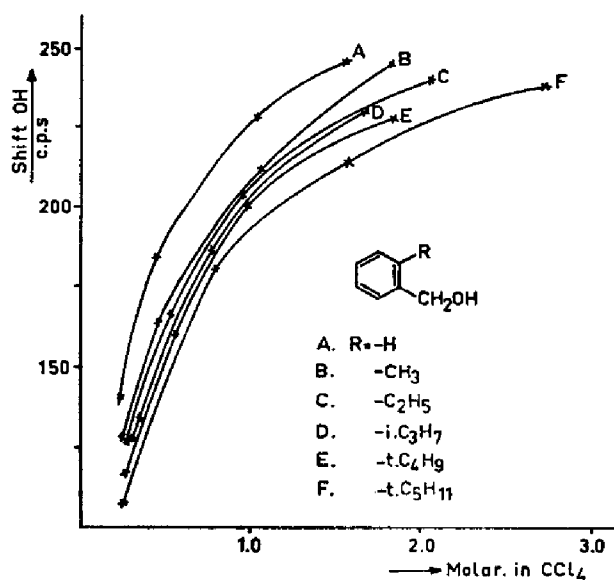
fig. 4   Concentration dependency shift-OH proton in benzylalcohols.

cyclohexanols, all of which are known to be almost exclusively present in the chair position.

2   The aromatic group of all compounds listed in table 6 is for the greater part oriented equatorially. This is indicated by the signal of the hydroxyl proton.

3 The aromatic group of all compounds listed in table 5 is mainly oriented axially, a fact which is also shown by the signal of the hydroxyl proton.

The compounds in table 5 are identical to those listed in table 6 but for the fact that in table 5 the ortho substituent is invariably a tertiary alkyl group.

4.2 THE CONFORMATION AT C_2

At the C_1 atom the compounds in table 6 show a conformation which agrees to the one forecasted on the basis of the concept of the steric approach control, if we allow the assumption that no post-reaction ring inversion has occurred.

The compounds listed in table 5 show a conformation at the C_1 atom which does not agree to the concept mentioned, if the same assumption as above is made. But, the supposition that the ortho-tert.alkylphenyl-Li reagent should not approach the carbonyl function from the least hindered side would appear highly improbable in the face of the behaviour of all other phenyl-lithium reagents.

It must be concluded, therefore, that, since one does have a conformation at C_1 with an equatorial hydroxyl group, while the axial approach leading to this conformation can be excluded, ring inversion offers the only possible explanation.

We do suggest therefore that initially ortho-tert.alkyl-phenyllithium compounds also yield products with an axial hydroxyl-group, but that these are subsequently subject to ring inversion. Model considerations show that an ortho-tert.alkyl group in an equatorially oriented phenyl nucleus is subject to a considerable interaction with the equatorial substituent in the 2-position of the cyclohexane ring; even a H-nucleus may, although in a lesser degree, be responsible for such an interaction.

In compounds with primary or secundary alkyl substituents in the benzene ring, the alkyl substituent may assume such a conformation, that interaction with the 2-substituent in the cyclohexane ring is much reduced.

No such an interaction occurs if the ortho-tert.alkyl phenyl group is axially oriented. And although even so the presence of the 3- and 5-cis axial hydrogens precludes completely free rotation, it is this

conformation which would seem to be most favoured (fig. 5). Recently, Siccic and Welvart[105] concluded on the basis of P_k values that in 1-N-dimethylamino-1-phenylcyclohexane the conformation with axial phenyl group is favoured. The cause for this would be the hindrance of the free rotation of an equatorially oriented phenyl group by a geminal dimethylamino group. As stated before, the conformation of the 1-tert.alkylphenylcyclohexanols is suggested to be the result of a ring inversion of the initially formed compounds.

The equilibria mentioned on page 27 will lie far to the right for compounds with these aryl groups.

——OH   ——o CH₃   ——• R
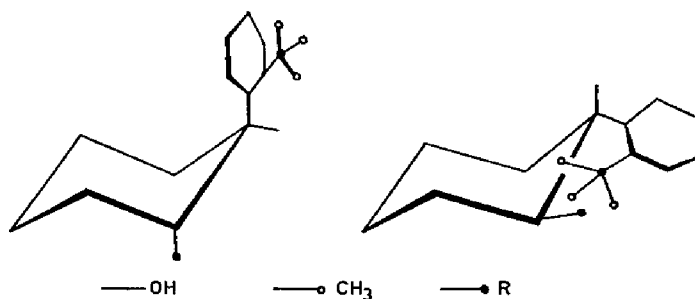
fig. 5   Steric hindrance in trans-2-alkyl-1-(ortho-tert.butylphenyl)cyclohexanols.

Obviously the ring inversion has also changed the conformation at C_2. In the case of the 2-methyl and 2-ethyl cyclohexanones we may assume that the conformation with an equatorial alkyl group is the only one present.

In cyclohexanols with an ortho-methyl, -ethyl or -isopropyl group in the phenyl nucleus, the 2-alkyl group will be oriented equatorially, in the ortho-tert.alkylphenylcyclohexanols axially. This will bear its consequences for the signal of the 2-alkyl group in the NMR spectrum. In general the differences for cis or trans situated alkyl groups are not important, but when two conformations are to be compared, they appear nevertheless as sufficiently significant.

Cocker et al., for instance, report for the CH_3 signals of some isomeric terpenes the following values: CH_3 axial — 0.95, CH_3 eq. — 0.85 and CH_3 ax. — 0.91, CH_3 eq. — 0.86, and also in the same order 0.82 and 0.70; all values in ppm unities (δ) with regard to $Si(CH_3)_4$.[25]

The resonance of the equatorially oriented CH_3 group occurs in all cases at the highest field; the δ-value $\pm$ 0.9 ppm, according to Cocker, is identical to the one in methylcyclohexane, while according to our own measurements in 2-methylcyclohexanone, the resonance occurs at 0.92 ppm.

The resonance values of the —CH_3— and of the —CH_2CH_3— protons in our compounds are summarized in table 12.

TABLE 12

Relation between conformation and shifts in several 2-alkyl-1-(ortho-alkylphenyl)-cyclohexanols

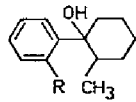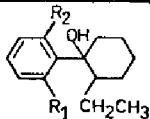| R	$\delta\,CH_3$	$\delta\,OH$	R_1	R_2	δCH_2CH_3	$\delta\,OH$
CH_3	0.64	1.35	CH_3	H	0.81	1.47
C_2H_5	0.66	1.47	C_2H_5	H	0.81	1.40
$i-C_3H_7$	0.67	1.33	$i-C_3H_7$	H	0.84	1.38
			CH_3	CH_3	0.84	1.46
			C_2H_5	C_2H_5	0.81	1.45
$t-C_4H_9$	0.58	1.77	$t-C_4H_9$	H	0.75	1.77
			$t-C_5H_{11}$	H	0.73	1.72

The compounds below the line in table 12 show the resonance of the 2-alkyl group positions at a lower δ-value than the other ones; these alkyl groups are oriented axially.

This does not agree with the rule that an equatorially oriented alkyl group should resonate at the higher field.

Although literature indicates that the influence of the hydroxyl group may be neglected, a β-OH group showing an almost complete lack of effect on the shift of the protons of the alkyl groups, in our case an effect of this group on the methyl group can definitely be noted.[62]

Eliel and Biros report for trans-2-methyl, cis-3-methyl and trans-4-methylcyclohexanol, in which the hydroxyl group and the methyl group are always equatorial, shifts of respectively 0.98, 0.94 and 0.89 ppm downfield with regard to TMS [a]).[42] Evidently there is a deshielding effect.

---

[a]) The authors report c.p.s. units which we converted in the δ-values.

From the spectra of trans-2-methyl-trans-4-tert.butyl- and cis-2-methyl-trans-4-tert.butylcyclohexanol, in which compounds the hydroxyl group is oriented equatorially and the methyl group in the first case equatorially and in the second axially, it appears that the resonance of an axial methyl group occurs at a higher field (δ respectively 0.96 and 0.92) [a]).

A hydroxyl group in 2 position in α-methylcyclohexanes therefore causes inversion of the shifts of the methyl groups with regard to the situation in compounds where no hydroxyl group is present.

In the case of trans-2-methyl-cis-4-tert.butylcyclohexanol the δ-value is also 0.92 ppm (hydroxyl axial and methyl also axial). A phenyl group situated at the neighbouring carbon atom is of greater importance however. The effects caused are large; compare for instance the values (hydroxyl) reported by Ouellette (see page 31) for trans-4-tert.butylcyclohexanol with those obtained by Carr (see page 30) for trans-2-tolylcyclohexanols.

A similar effect was obtained by Casey for the signals of the 3-methyl group in cis and trans 1,3-di-methyl-4-phenylpiperidinol-4.[22] It is still not very clear as to what will be the differences between the methyl signals for the di-equatorial position and the di-axial position of the methyl and the phenyl group.

Which position the ortho-tert.butylphenyl group will take exactly is not known, but a purely axial position is not probable. Application of the rule of Johnson-Bovey for the screening by the benzene ring is not possible.[63]

The marked difference in both groups of compounds in table 12 cannot be caused by for example varying screening influence of the benzene nucleus in different rotation positions of this nucleus[22]

The difference is convincing proof for the correctness of the concept of the steric approach control. The ring inversion which occurs in the second group of table 12, influences both the shift of the hydroxyl group and that of the 2-alkyl group. This inversion may be considered as a product development control.

In fig. 6-9 some NMR spectra have been depicted; they show an identical picture for the cyclohexyl part of all compounds. The differences, observed in the case of the ortho-tert.butyl derivative are very striking (signal hydroxyl proton).

---

[a]) The authors report c.p.s. units which we converted in the δ-values.

38

The signal of the aromatic protons might also be expected to show differences depending on whether the aromatic group is axially or equatorially oriented with respect to the cyclohexane nucleus. It proved impossible, however, to determine the exact resonance value of the aromatic protons, because of the wide spread. This was in particular true for the tert.butyl derivative (fig. 9). A similar broadening is also noted in the spectrum of tert.butyl benzene and it seems probable, therefore, that anisotropy is caused by a screening effect, for instance, of the large group. Although to a lesser degree, the isopropyl group has the same effect. In the literature this phenomenon has been described for several alkyl benzenes, as well as for biphenyl.[10]

It would have been very interesting to learn in what way the product to be obtained by reaction of 2-tert.butylcyclohexanol and 2-tert. alkylphenyl-lithium would react to ring inversion. However, all efforts to obtain 1-aryl-2-tert.butylcyclohexanols, by reaction of 2-tert.butylcyclohexanone, with either ortho-isopropyl or with ortho-tert.butylphenyl-lithium, failed completely.
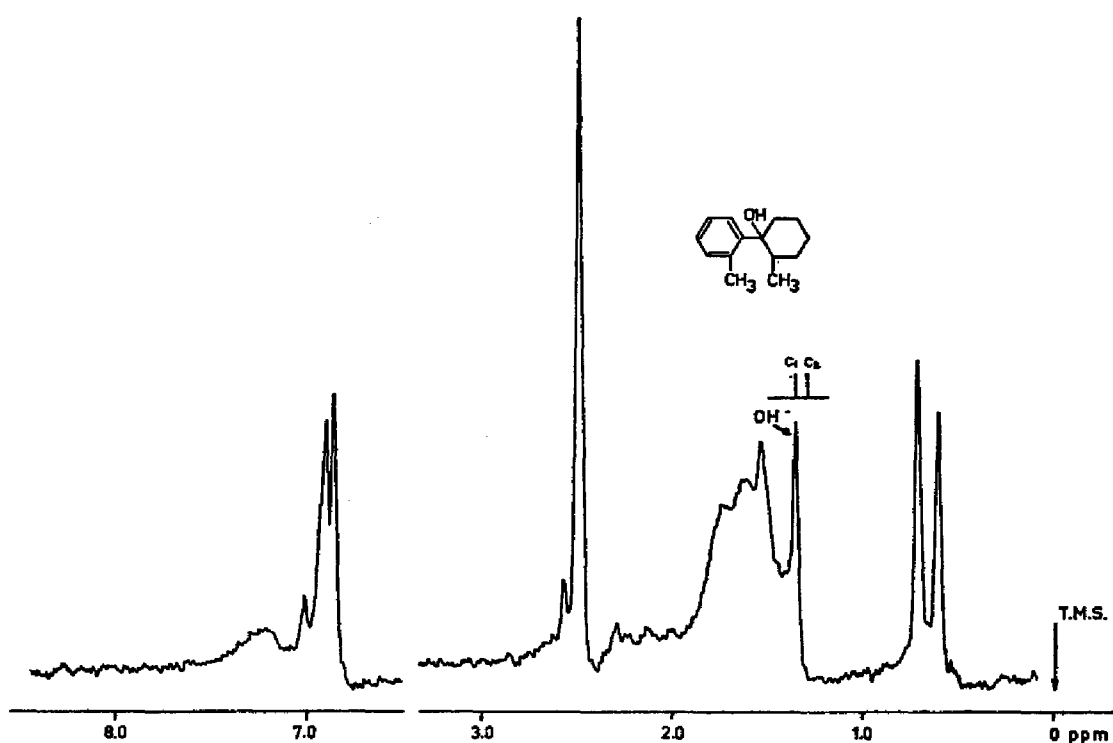
fig. 6   NMR spectrum of 2-methyl-1-ortho-tolylcyclohexanol. The positions (C_1, C_2) show the OH signal at the concentrations; $C_1 = 2C_2$.
C_1 0.75 mol in CCl_4.
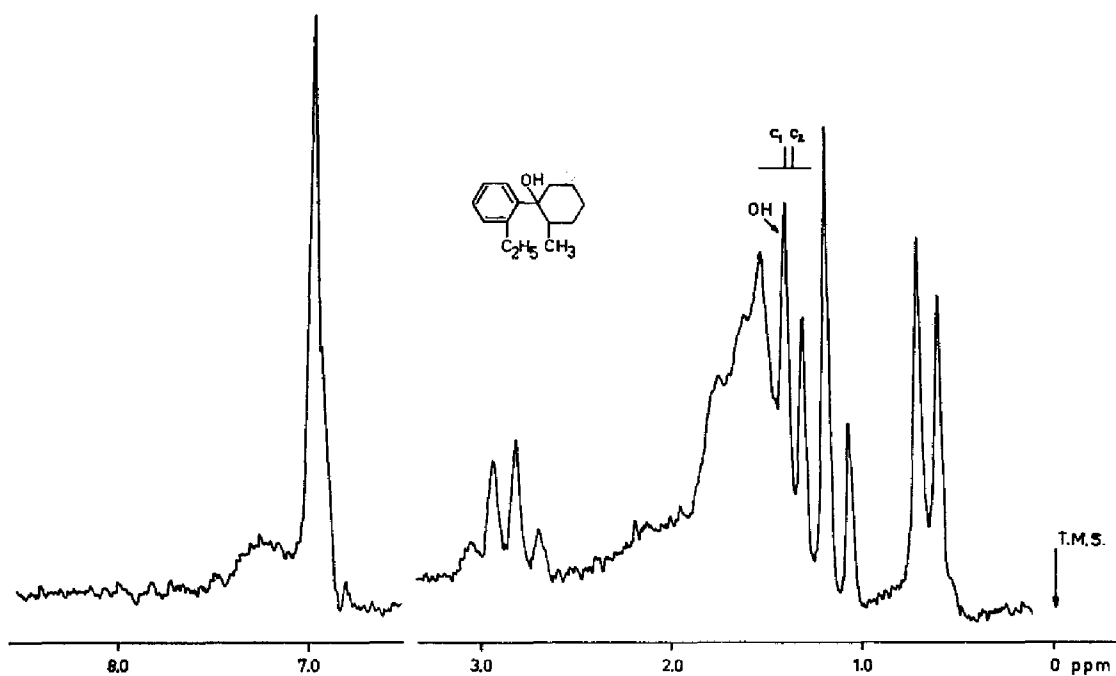
fig. 7   NMR spectrum of 1-(ortho-ethylphenyl)-2-methylcyclohexanol. For more details see fig. 6.
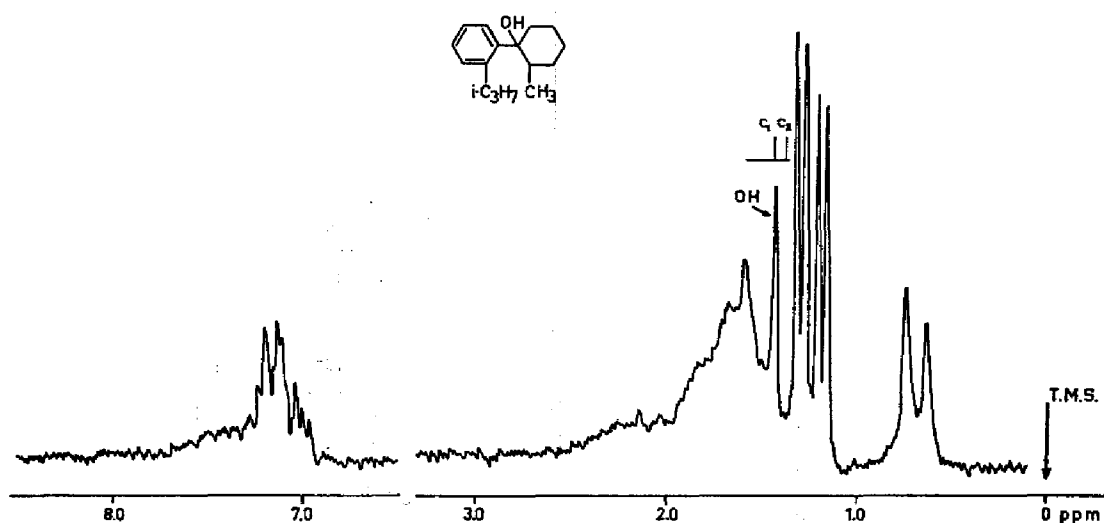
fig. 8   NMR spectrum of 2-methyl-1-(ortho-cumenylcyclohexanol. For more details see fig. 5.[a]

---

[a]) The occurrence of isopropyl-CH_3 as a quartet in stead of a doublet and the relation to the presence of an asymmetric carbon atom in the molecule has been described by v. d. Vlies, Rec. Trav. Chim. 84, 1289 (1965).

40
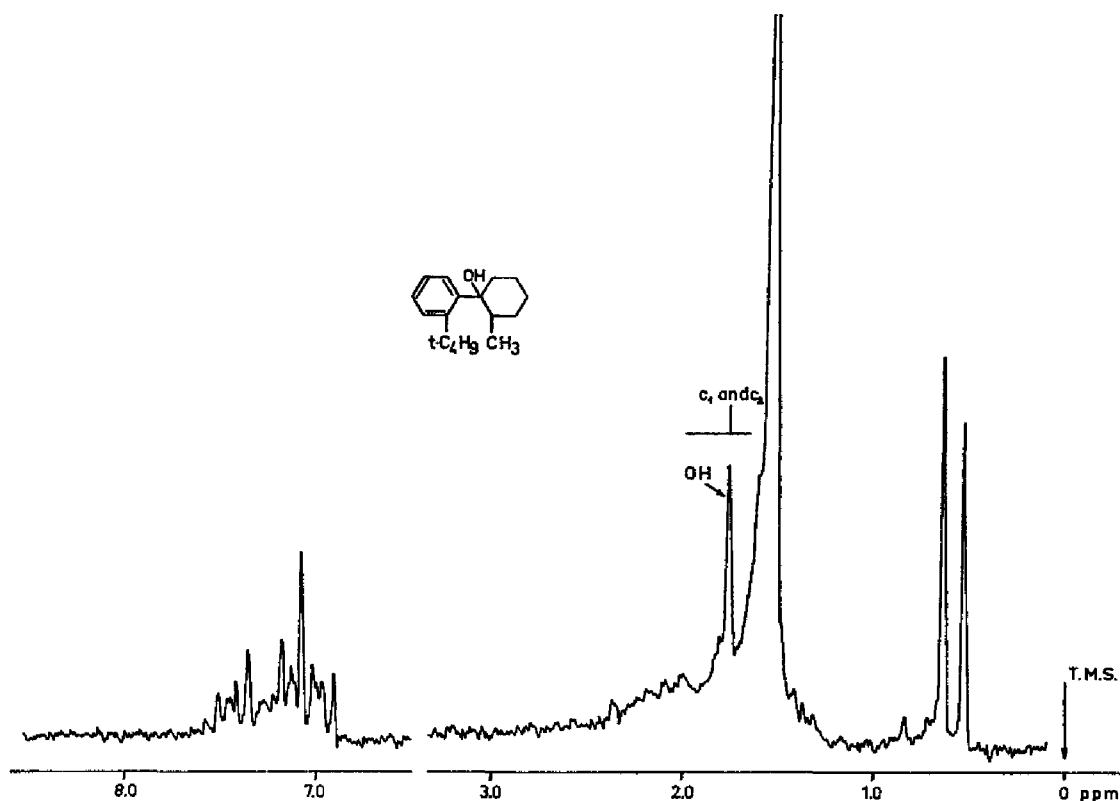
fig. 9 NMR spectrum of 1-(ortho-tert.butylphenyl)-2-methylcyclohexanol. For more details see fig. 6.

We tried several times, each time under different reaction circumstances, but in none of the cases we did obtain an arylcyclohexanol. This was definitely determined by both IR and NMR spectra of both distilled fractions and reaction residues.

Invariably we obtained almost exclusively the unaltered ketone and a substituted benzene.

The total failure of the synthesis can probably be ascribed to the steric hindrance in both reaction components. As shown in tables 1 and 2, the yield of the reaction depends very much on the substituents in each of the ring systems and it is especially low if one of the substituents happens to be a tertiary alkyl group.

Particularities about the reaction circumstances of the various attempts to synthesis may be found under the heading "Syntheses".

41

4.3 SYNTHESES

A   Ortho-alkylbenzylalcohols

The synthesis of the acids, which were used as starting materials, was carried out according to commonly known methods. The acid chloride was synthesized from the acid with the aid of thionylchloride. In particular where there is a considerable steric hindrance by the alkyl substituent, difficulties arise here. However, by profiting from the catalytic properties of DMF in this reaction, all acid chlorides may be easily obtained.[9] In the reduction of the acid chlorides to the benzylalcohols with the aid of LiAlH$_4$, high yields are obtained by using the method described by Field and Grundy for the reduction of both acid chlorides and esters.[46]

In table 10, page 33, the data of the benzylalcohols are reported; in table 13 are summarized the data of the intermediate products.

TABLE 13

Some data of ortho-alkylbenzoic acids and the corresponding acid chlorides

ortho-alkyl substituent	acid		acid chlorides	
	yield from the bromo-benzene	M.P.	yield from the acid	B.P.
ethyl	86 %	—ᵃ⁾	74 %	98-100/15
i–propyl	70 %	59°	82 %	116/28
t–butyl	43 %	68°	70 %	104/10
t–pentyl	63 %	—ᵃ⁾	82 %	124-129/16

ᵃ⁾ Not established, as the available amounts did not allow extensive purification.

B   2-Alkyl-1-(ortho-alkylphenyl)cyclohexyl-para-nitrobenzoates

As all known methods of esterification failed – tosylates, for instance, could not be obtained in any possible way – and as it seemed nevertheless desirable to obtain derivatives of the cyclohexanols, we proceeded to synthesize some p-nitrobenzoates in the following way:

The Li-alcoholate of the carbinol – of which an excess is used – is prepared with the aid of phenyllithium.[21] To this alcoholate is added an acid chloride.

General procedure:
Phenyllithium was prepared from 0.03 moles of bromobenzene with Li metal, after having led dry N_2 through the thoroughly dried apparatus for about 45 minutes.

By means of N_2 pressure all phenyllithium – in ethereal solution – is forced into another reaction flask via a communicating glass tube which is provided in the widened bottom end with a wad of glass wool which serves as a filter. Li rests and the precipitated LiBr remain.

0.035 moles of 2-alkyl-1-(ortho-alkyl)phenyl-cyclohexanol are added to the solution of phenyllithium. The reaction which follows is violent and after the mixture has been stirred for about 30 minutes, a solution of 0.03 moles of p-nitrobenzoylchloride is added.

Again a violent reaction is observed during which a precipitate of LiCl is formed. The mixture obtained is finally boiled during one hour. The precipitate is then filtered.

The solution is evaporated and the residue is taken up in petroleum ether (60-80°).

The insoluble part – which mainly consists of p-nitrobenzoic acid – is separated. The ester crystallizes from the petroleum ether. Recrystallization from the same solvent affords the pure ester.

The yields are low, (30-40 %).
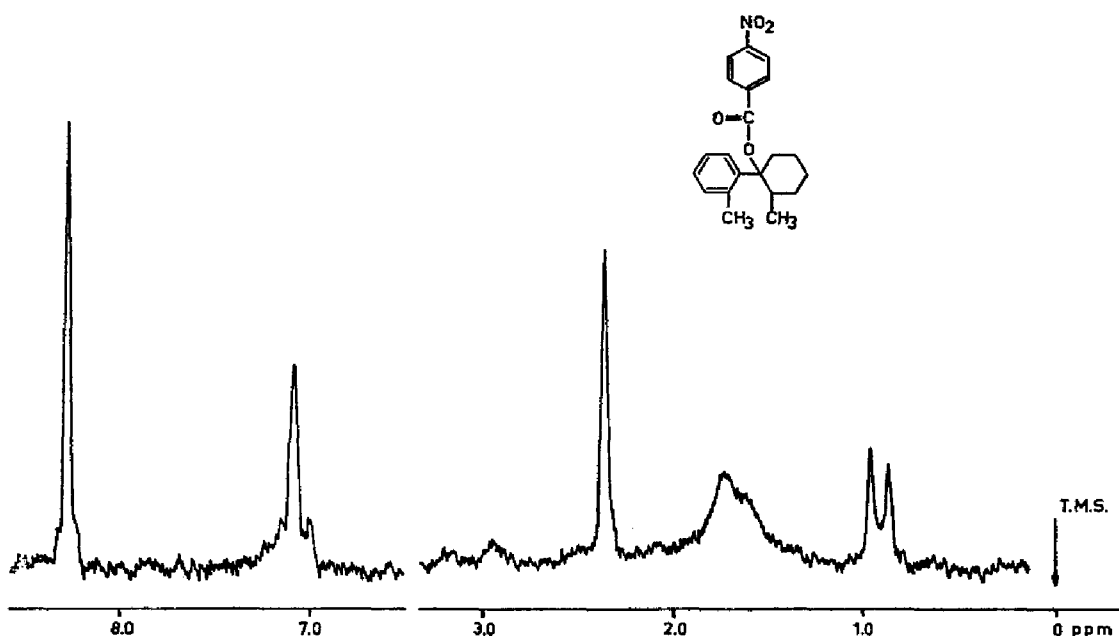
fig. 10 NMR spectrum of 2-methyl-1-ortho-tolylcyclohexyl-p-nitrobenzoate. 10 % in CCl_4.
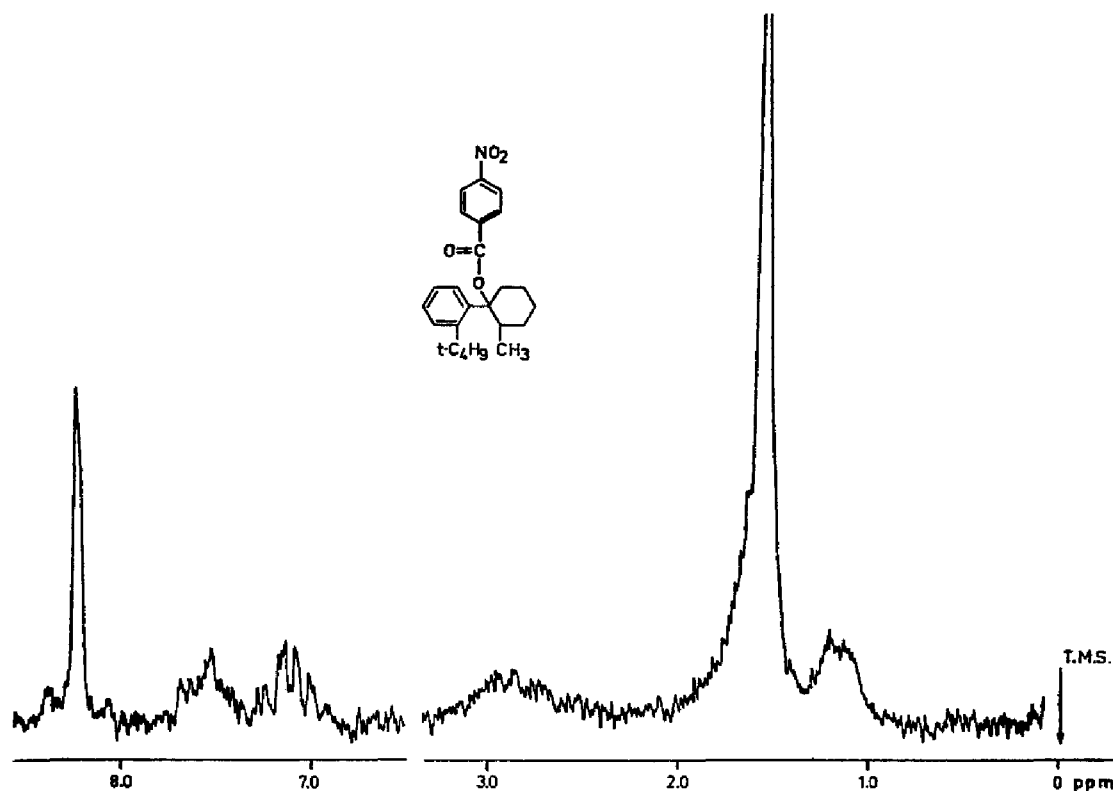
fig. 11   NMR   spectrum   of   1-(o-tert.butylphenyl)-2-methylcyclohexyl-p-nitrobenzoate. Saturated solution in CCl_4.

The following 1-aryl-2-methylcyclohexyl-p-nitrobenzoates were prepared:

Aryl = ortho-totyl $C_{21}H_{23}NO_4$, 353.28
                              C 71.42, H 6.53, N 3.97
                  found   C 71.7   H 6.5   N 3.9)
Aryl = ortho-ethylphenyl $(C_{22}H_{25}NO_4$, 367.31
                              C 71.90, H 6.85, N 3.81
                  found   C 72.4   H 6.8   N 3.8)
Aryl = ortho-tert. butylphenyl $(C_{24}H_{29}NO_4$, 395.36
                              C 72.91, H 7.37, N 3.55
                  found   C 72.8   H 7.4   N 3.6)

All these esters are of a yellow colour and poorly soluble in the common organic solvents. The IR and the NMR spectra do agree with the structure (see fig. 10, 11).

44

C Summary of the efforts towards the synthesis of 2-tert.butyl-1-(ortho-tert.butylphenyl)- and of 2-tert.butyl-1-(ortho-isopropylphenyl)cyclohexanol

1 The general method as described on page 19 yields only the starting products, except for the orthoalkylbromobenzene, instead of which we did obtain the debrominated product. There is a slight residue, but according to its IR spectrum this does not contain any cyclohexanol.

2 As under 1 but after addition of the ketone to the phenyllithium reagent, PE 100-120° was substituted for ether as a solvent; the reaction mixture was boiled for about 15 hours this time.

3 Instead of ether (diethyl) we used diglym (= dimethylether of diethylene glycol) as a solvent. The general method was again used; temperatures used were naturally higher. The results obtained at 2 and 3 are identical to that under 1, but for a larger amount of residue.

4 As under 2, but the ether, instead of being replaced by petrol-ether, was substituted by diglym, during the later stages of the procedure which did not, however, improve the result.

5 In view of the possibility of a reaction between the Li metal and the solvent, and hence a subsequent reaction between the formed lithium alkyl compounds with the ketone, the phenyllithium reagent was prepared in the apparatus mentioned on page 43 in ether.
   It is then forced into another flask by means of N_2 pressure, after which the ether is replaced by diglym. We then proceeded to the addition of the ketone. The intended result was however not obtained.

6 Instead of using Lithium we used the Mg-Grignard compound. Reaction circumstances were as described under 1.
   We did not obtain any phenylcyclohexanol.

The various reactions mentioned were repeated several times. At no time did we succeed, however, to isolate the desired product.
In the distillable fractions we obtained but the basic products; the reaction residue did contain only unidentified products which were evidently different from cyclohexanol.

CHAPTER 5 REACTIONS WITH CONFORMATION-
HOMOGENEOUS COMPOUNDS

5.1 INTRODUCTION

In conformation analyses of cyclohexane derivatives, one often uses compounds with a stable ring conformation which is not subject to inversion.[44] Most frequently used for this purpose are either a 4-tert.butylcyclohexane or a trans decalin derivative.

Synthesis of 2-alkyl-1-arylcyclohexanols from 2-alkylcyclohexanones and an ortho-alkylphenyllithium compound may, as we have seen in the preceding chapters, occasionally be accompanied by ring inversion; this inversion takes place when a tertiary ortho-alkyl substituent is present in the aryllithium compound. The use of a cyclohexanone which yields a cyclohexanol which is unable to undergo ring inversion, might therefore afford an argument to prove the correctness of the suggested reaction course.

5.2 4-TERT.BUTYL-1-(ORTHO-TERT.BUTYLPHENYL)-
CYCLOHEXANOL

The choice of 4-tert.butylcyclohexanone was dictated by the fact that steric hindrance prevents its 2-analogue to form the desired product from the reaction with ortho-tert.butylphenyllithium.

Synthesis 4-tert.butylcylohexanone.
The 4-tert.butylcyclohexanol was obtained by hydrogenation of p-tert.butylphenol (reaction circumstances as for the ortho analogue, see page 17). Without purification the product is next oxydized with sodium bichromate.[15]
The ketone obtained is crystallized from petroleum ether (40-60°). The melting point, 50°C, is in agreement with the corresponding data found in the literature.[104]

Synthesis of 4-tert.butyl-1-(ortho-tert.butylphenyl)cyclohexanol.
The synthesis was carried out as described under 1, 2, 3 and 4, on page 45.

46

Notwithstanding the fact that in each of these cases a distinct reaction could be seen, which was not surprising in view of the absence of steric hindrance in the ketone, we did not obtain any single products. The fractions obtained by distillations showed considerable differences in their IR and NMR spectra, all of which indicated, in addition, that the desired compound was not present at all. Apparently some unsaturated compounds had been formed as well as breakdown products of the desired substance.

A further indication for the absence of the desired cyclohexanol was found in the boiling points of the various fractions. In one synthesis for instance distillation resulted in the following fractions:

1 boiling point 122°-123°, 10^{-1} mm Hg
2 boiling point 127°-128°, 10^{-1} mm Hg

NMR spectra proved that fraction 1 contained several products which possess no cyclohexane nucleus, whereas in fraction 2 there was an almost total absence of aromatic groups. We refrained from further identification.

The destruction which evidently has taken place may have occurred at the high reaction temperatures or in the course of the distillation. For this reason diethylether was subsequently used for the reaction and distillations were omitted.

Since isomeric cyclohexanols may be separated by chromatography, the crude reaction mixture was absorbed on silicagel and eluted with benzene-ether-petroleum ether (1 : 1 : 1). The oily cyclohexanol compound was eluted first, followed by the ketone. The obtained cyclohexanol is not altogether pure (> 90 %, GLC analysis), it is contaminated by several rather volatile compounds.

According to the GLC the main fraction consists of two components which could not be separated completely. The minor one which constitutes about 10 % of the main fraction, shows the longest retention time.

Conformation

The NMR spectrum reasures us as to the identity of the desired compound. It shows a second peak at a slightly higher field than that of the aliphatic tert.butyl group signal.

This does *not* originate from possibly carried-over 4-tert.butyl-cyclohexanone. The integral of this supplementary peak constitutes ± 10 % of that of the mentioned tert.butyl group. It is very probable that this signal is emitted by the minor component of the

main fraction. Its position indicates that it might also originate from a tert.butyl group.

Table 7 (page 23) shows that introduction of a substituent in the 2-position of the cyclohexanone ring considerably increases selectivity. A lowering of the selectivity as a result of the absence of the 2-substituent may well have led to the presence of two isomers in the case of the reaction of 4-tert.butylcyclohexanone with phenyl-lithium compounds.

The fact that the resonance of the 4-tert.butyl group in A occurs at a slightly higher field than that of B, should be attributed to the shielding effect of the phenyl nucleus in cis-position (fig. 12). The resonance of the hydroxyl proton – main component B – takes place at $\pm$ 1.30 ppm; this is in agreement with an axial orientation of the hydroxyl group and with the shortest retention time.
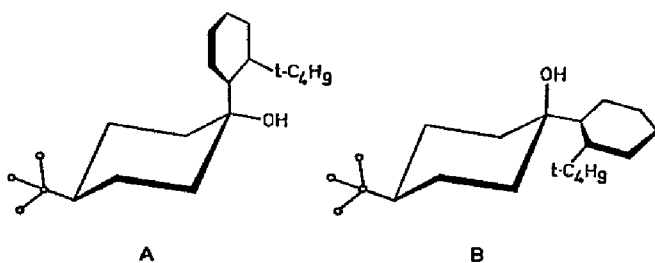
fig. 12   Cis (A) and trans (B) 4-tert.butyl-1-(ortho-tert.butylphenyl)cyclohexanol.

Because of the impossibility of ring inversion there has been mainly equatorial addition. This would be in conformance to the steric approach control and to the reaction course as suggested by us.

The cis-form (fig. 12A) has resulted from axial approach and constitutes the minor component.

We failed to identify any signal of the hydroxyl proton of A in the NMR spectrum. In view of the slight concentration of A this is not surprising. The size of the group in the vicinity of the hydroxyl proton prevented an effective chromatographic separation of the mixture into its components.

Gas-liquid chromatography carries the disadvantage that a high temperature is required.

48

5.3 1-ARYL-TRANS-DECALOLS-1

The trans decalon was chosen for its propensity to engender conformation-homogeneous carbinols and further because of its bulky substituent in the 1-position.

Synthesis trans-α-decalon.

Hydrogenation of α-naphtol results in a mixture of the various decalols; its composition depends on the reaction circumstances during the hydrogenating[76 43 58]. Subsequent oxydation with chromic acid yields pure trans-α-decalon; since the cis-α-decalon isomerizes into the trans product under the circumstances of the oxydation reaction[58]. Besides, it has been reported that boiling with acid-methylalcohol is able to transform the cis- into the trans-isomer[37 77]. The hydrogenating of the α-naphtol was carried out according to the method indicated by Birch[7]. Hydrogen appeared to start being absorbed at lower temperatures than reported by this author. However, the amounts absorbed are in that case not equimolecular. Fresh absorption of H_2 may be induced by subsequent raising of the temperature. In the light of the foregoing we are inclined to think of an initial formation of tetrahydrogenated products.

The obtained carbinol mixture, from which any possibly remaining naphtol has been eliminated by washing with alkali, is thereafter oxydized according to Brown[75].

The product obtained proved a mixture of cis-trans decalon, with probably also slight amounts of tetralon. The cis-α-decalon was converted into the trans isomer, by boiling with acid-methylalcohol for two hours. We obtained a product which boiled at 63-64° (1 mm); (Djerassi 40/0.1). It solidified and crystallization from PE 100-130 yielded the pure trans-α-decalon which according to GLC analysis contained less than 1 % impurities.

The melting point lies just above room temperature and is difficult to determine. The product obtained was identical to a sample of authentic trans-α-decalon (IR, NMR).

Synthesis 1-phenyl-trans-α-decalols.

The synthesis is identical to that of 1-phenylcyclohexanols. The yields obtained are of the same order.

In table 14 the data of the synthesized products have been summarized.

The ortho substituted phenyldecalols are, without exception, single products. According to GLC analysis no isomers have been formed. The unsubstituted 1-phenyl-trans-α-decalol, however, consists of two isomers.

The ratio of their respective amounts in the reaction mixture is 7 : 1 (GLC).

49

TABLE 14

Some data on
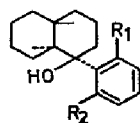
R_1	R_2	boiling point	N_D^{20}	elem. analysis				yield from trans-α-decalon	NMR; δOH; 0.6mol	
				$C_{calc.}$	C_{found}	$H_{calc.}$	H_{found}		in CCl_4	in $CDCl_3$
H	H	99-101/10⁻²	1.5493	83.48	83.7	9.57	9.6	80 %	1.65 [a]	1.72
CH_3	H	100-105/10⁻²	1.5537	83.61	83.6	9.84	9.9	70 —	± 1.65 [b]	1.75
CH_3	CH_3	138-140/10⁻¹	1.5541	83.72	83.4	10.08	10.1	40 —	± 1.75 [b]	1.95
t-C_4H_9	H	120-124/10⁻¹	— [c]	83.96	84.0	10.49	10.5	49 —	1.83	2.00

[a] Measured on mixture of cis-trans and trans-trans (see text); signal main component.
[b] The values cannot be accurately determined; the signal of the hydroxyl proton in a 0.6 molar solution is obscured by other signals.
[c] Solid; m.p. 64.5-66.

Judging by the retention times we may conclude that the isomer, present in excess, carries the hydroxyl group axially.[48]

An identical ratio has been reported by Smissman et al. for the mixture resulting from the reaction between phenyllithium and 1-methyl-trans-4-ketodecahydroquinoline.[90]

It proved impossible to execute a quantitative separation of the components (GLC). Distillation of the mixture by means of a Claisen flask did not change the isomer ratio.

Conformation

We did not find any descriptions of 1-phenyl substituted trans-α-decalols in the literature.

The unsubstituted products however have been investigated exhaustively, e.g. by Hückel.[58] The literature also mentions the NMR spectra of the unsubstituted decalols.

Feltkamp et al. were able to identify both trans-α-decalols, again by means of the resonances of the α-protons.[45]

Unfortunately they do not report the concentration in $CDCl_3$, at which these spectra were taken and which is of great importance for the signal of the hydroxyl proton. The difference between an axially and an equatorially oriented hydroxyl group again is reflected in resonance occurring at higher field for the axial orientation.

50

If it is now presumed that both decalols were measured in identical concentrations, the distance between the positions of the signals is of about the same order as for axially and equatorially oriented hydroxyl groups in cyclohexanols.

We performed NMR spectra of all compounds which we had synthesized both in CCl_4 and in $CDCl_3$, – molarity in either solvent 0.6. Once again the signal of the hydroxyl proton distinguishes itself by a slight shift occurring when the sample is diluted.

The data found are recorded in table 14.

If we now are permitted to assume that in the first three compounds in table 14 the phenyl group is oriented equatorially, as is the case in the corresponding cyclohexanols, we are surprised to find that the resonance of the hydroxyl protons all occur at δ-values which are situated 0.2-0.3 higher than those of the 1-phenylcyclohexanols. The variation in the series is of the same order.

The cause of this downfield shift may be presumed to lie in the shielding effect of the benzene nucleus; see below for the 1-(2.6-dimethyl)phenyl-α-trans-decalol.

Increase of the size of the substituent causes a shift towards lower field, a phenomenon also witnessed for the cyclohexanols, in which the 2-substituent is of determining influence.

In the case of the cyclohexanols the transition of ortho-isopropyl to ortho-tert.butyl substitution in the phenyl group is attended by a sudden jump in the shift of the hydroxyl proton of about 0.35-0.40 ppm unities; this is due to ring inversion in the mentioned compounds.

In the case of the decalols, increasing bulk of the substituent causes a more gradual transition to resonance at lower field. There is no sudden change for the ortho-tert.butylphenyl derivative.

The interaction between the ortho-alkyl substituent in the phenyl nucleus and the substituent in 2-position on the cyclohexanol is less than the corresponding interaction between the ortho-phenyl substituent and the α-CH_2 in the adjacent six-carbon atom-ring in the 1-phenyl-trans-1-decalols.

This is clearly demonstrated by the fact that the two methyl groups in 1-(2.6-dimethylphenyl)-trans-1-decalol, gave rise to two signals in the NMR spectrum; the separation is about 5 cps unities; in the corresponding cyclohexanol one signal is shown.

Rotation of the phenyl nucleus will therefore be inhibited and its position rather fixed. The benzene nucleus will be oriented almost

vertically to the plane of the saturated ring.[22] The difference between the signals of equatorial and axial hydroxyl groups is at least 0.35 ppm (concentration 0.75 molar) in the cyclohexanols we investigated; in the case of the unsubstituted decalols Feltkamp reports a value of 0.42 ppm (if the concentrations of both decalols are taken to be identical). These differences and the ones we observed indicate that the 4 synthesized decalols are all present in the same conformation.

In view of literature data on the selectivity of this kind of reactions (see table 7, page 23) and the results obtained with the corresponding cyclohexanols, it may be concluded that the phenyl group is always oriented equatorially in the case of the 4 decalols of table 14 (in the unsubstituted phenyl derivative the main component is meant, invariably).

They are formed, without exception, by equatorial approach of the phenyllithium compound by conformance to the concept of the steric approach control and the reaction course we suggested. The results obtained by us during the synthesis of these conformation-homogeneous carbinols have proved the correctness of our explanation of the anomalous conformation of 1-(ortho-tert.alkylphenyl) cyclohexanols. Apparently this conformation is not due to the carbonyl function being axially approached, since in the series we have now synthesized no deviating behaviour of the ortho-tert.butylphenyl compounds was encountered.

SUMMARY

The conformation of cyclohexane derivatives has in the last years been the subject of a great number of investigations. General rules, usually applied to predict selectivity in the case of additions to ketones, proved inoperative in the case of cyclohexanones. For these compounds other rules have been derived, a.o. by Dauben; they were most often based on reduction reactions.

Selectivity was thought to be determined by both steric and energetic factors.

In the present investigation we demonstrated, using literature data, that in reactions between cyclic ketones and organo metallic compounds such as the Grignard reagents and Li-compounds, selectivity is governed by steric factors.

A growing crowding round the metal atom of the organo-metallic reagent and/or the carbonyl function in the ketone, will provoke a proportional growing affinity for equatorial approach (least hindered side) by the metallic reagent to the carbonyl function. The hydroxyl group formed by equatorial approach will consequently have an axial orientation.

The configuration of the single isomer which according to GLC analysis is obtained by reaction of ortho alkyl-phenyllithium compounds and 2-alkylcyclohexanones, could be forecasted with the aid of the above enumerated data.

By means of NMR spectra of the 2-alkyl-1-(ortho-alkylphenyl)-cyclohexanols obtained we were able to prove the correctness of our theory with regard to the formation of the trans-isomer.

Although, in the case of the ortho-tert.alkylphenyl compounds, the expected conformation with an axial hydroxyl group did not arise – the position of the hydroxyl proton signal having been compared with literature data – the remaining cyclohexanols did show the expected conformation. It could be proved, however, by means of the signals of the substituents in 2-position on the saturated nucleus that the products obtained, in the case of the tert.alkyl derivatives, are nevertheless trans-isomers, but that the equatorial approach had been followed by ring-inversion. The ring-inversion is the result of a strong interaction between the ortho-tert.alkyl group and the substituent in 2-position on the cyclohexane ring, an interaction

which is not encountered in the case of a primary or a secundary ortho-substituent.

This phenomenon can be demonstrated with the aid of models. Whenever ring-inversion is impossible, that is whenever the cyclohexane ring is compelled by its substituents to adopt only one definite conformation, as for instance in the case of tert.butyl substituted cyclohexyl and trans-decalyl compounds, we can indeed observe a conformation of the ortho-tert.butylphenyl derivatives which is identical to the one shown by corresponding compounds without tertiary ortho substituents.

PART II PHARMACOLOGICAL INVESTIGATIONS

CHAPTER 1 INTRODUCTION

The differences in conformation as described in the chemical part probably will have consequences on the biological activities of the compounds and therefore we have determined the biological activities of some series of this type of compounds.

Differences in activity found in experiments with intact animals may be caused by real differences in biological activity as well as by differences in metabolism or rate of excecretion. However, as the mean purpose of these pharmacological investigations is to correlate the conformation with the biological properties, the way by which the probable differences are induced, is *for these investigations* of minor importance.

Some phenylcyclohexanols show interesting biological properties. Several are reported to possess hypnotic properties, others have cardioplegic effects, e.g. some trans and cis 2-tolyl- and 2-chlorophenylcyclohexanols.[93, 91]

Of the isomer pairs the trans compound always proved to possess the stronger cardioplegic effect. This isomer carries both the phenyl and the hydroxyl group equatorially, whereas in the cis-isomer the hydroxyl group is oriented axially, and the phenyl group equatorially. The biological activities are therefore clearly related to the configuration of the compounds.

Among the compounds which we synthesized no isomer pairs occur.

However, we noted in some series a conformation transition of one chair position to the other.

In 2-methyl (and ethyl)-1-(orthoalkylphenyl)cyclohexanols with a tertiary alkyl, the aryl and the methyl (ethyl) will be oriented axially, and the hydroxyl group equatorially.

55

In the case of the aromatic groups o-tolyl, o-ethylphenyl or o-cumenyl, the entire situation is the reverse one.

The cardioplegic properties of the above mentioned 2-arylcyclohexanols will most probably have to be ascribed to the cyclohexanol group, with the phenyl group affecting the properties in dependence of the configuration.[59]

The difference between cis and trans 2-arylcyclohexanols lies in the conformation of one single substituent, and since the phenyl group is always predominantly present in the equatorial orientation, the difference between cis and trans is due to a different hydroxyl orientation, and as a result of the altered situation around the hydroxyl group cis and trans-2-arylcyclohexanols differ so markedly.

In the above mentioned series 2-methyl and 2-ethylcyclohexanols, alteration of the hydroxyl orientation affects the conformation of the molecule as a whole.

A change of o-cumenyl to o-tert.butylphenyl will cause the hydroxyl conformation to change from axial to equatorial.

The hydroxyl group will consequently be more exposed, which may be important for biological activity.

In the present case however, this effect is largely reversed by the increase in size of the geminal substituent.

It should be pointed out right from the start, that due to the bulky aryl groups and the resulting crowding round the biologically probably important hydroxyl group, chances, to observe clear differences correlating with the conformation may be expected to be low.

We investigated eight compounds in all, on different properties, viz. four substituted 2-methylcyclohexanols, and the corresponding four 2-ethylcyclohexanols, indicated with II, VI, X, XIX, and III, VII, XI, XX (page 19-20).

Mice were used to determine the toxicity of the compounds, as well as the influence on the metrazol threshold.

We also tested the activity of the substances on the coronary perfusion of the isolated guinea pig heart, both with and without previous administration of hypofyse extract to provoke coronary vessel constriction. The influence on the blood pressure of narcotised rats and cats was found to be minimal, hence hypertensive (renal) rats were used in additional investigations.

In order to determine how the excitability of the heart was influenced

by the cyclohexanols, we measured in several cases the strength duration curve of the isolated papillary muscle of the cat.

As some activities observed may possibly be caused via the β-sympathico receptors, we used the calf's trachea muscle, in order to determine whether the spasmolysis brought about by isoprenaline – the used β-sympathico mimeticum – in this preparation was affected by our compounds.

2.1 ACUTE LD$_{50}$ IN MICE AFTER I.P. ADMINISTRATION

The LD$_{50}$ was determined on mice [a]. In view of the poor solubility of the compounds, they were dissolved in cottonseed oil,[c] and administered intra-peritoneally (i.p.).

The LD$_{50}$ values [b] were based on the mortality 24 hours after administration; the cause of death was not established.

In fig. 13, the LD$_{50}$ values of the 8 compounds are expressed in thousandths of grammolecules per kg; the lines drawn are only meant to emphasize the course of LD$_{50}$ values in either series.
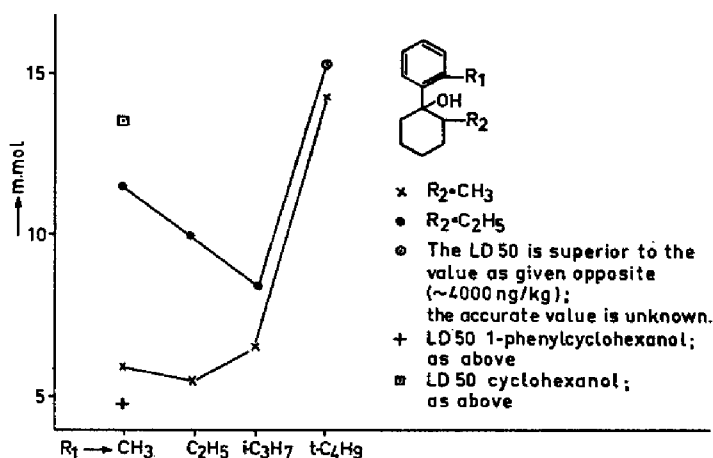
fig. 13 Acute LD$_{50}$ in mice, i.p. administration in cottonseed oil.

The relatively high LD$_{50}$ value of XIX and of XX is easily noted. These two compounds have a different conformation from the other six, (see chapter 4, chemical part).

In the 2-methylcyclohexanols the high value for the ortho-tert.butyl derivative (XIX) is less evident than in the 2-ethylcyclohexanols. It is however certain that the LD$_{50}$ depends on the orientation of the substituents at the cyclohexane ring. From the difference in the

---

[a] Swiss random bred - SPF.

[b] The LD$_{50}$ dose is the dose killing 50 % of the test animals.

[c] We used cottonseed oil for reason of the neglegible influence of this solvent such as contrasted with e.q. propylene glycol.

series it would appear that low LD_{50} values are a result of hemmed-in hydroxyl groups.

On the other hand, a "hindered" phenyl group will be favourable for a low toxicity. The high LD_{50} values for both o-tert.butylphenyl derivatives will hence well agree with an axial orientation of the phenyl group and the equatorial hydroxyl.

The unsubstituted 1-phenylcyclohexanol reveals at i.p. administration an LD_{50} in mice of 4,8 mmol/kg. We may presume in this compound a predominantly equatorial phenyl group and an axial hydroxyl. The compound does in both series indeed range among the relatively toxic ones.

An additional proof for an effect on the toxicity by the phenyl group is furnished by the LD_{50} of cyclohexanol itself, measured under the same circumstances (see fig. 13); it is 13,5 mmol/kg and cyclohexanol therefore ranges among the low toxic compounds as compared to the other investigated substances.

2.2 THIN-LAYER CHROMATOGRAPHY WITH REVERSED FASE

The differences in toxicity are possibly the result of variations in the resorption rates; these in their turn would be due to differences in lipid solubility.

Of all investigated compounds water solubility is very low, and a determination of, for instance, the partition coefficient of the compounds between water and an organic solvent, would for this reason be grossly inaccurate.

But use of thin-layer chromatography with 'reversed fase' renders it possible to determine the relative lipophilic character of members of a series of compounds.[11]

For this purpose the silicagel layer is impregnated with paraffin. The mobile fase is an acetone-water mixture.

A high Rf value indicates a hydrophilic character.

The Rf values of the eight investigated compounds were measured with 1-phenylcyclohexanol, of which the Rf value was put equal to 1.00, as a reference substance.

The results have been summarized in table 15.

The course of the Rf values in the two series does not correspond to that of the LD_{50} values: both series show a decrease of the Rf value, hence an increase of the lipohilic character.

TABLE 15

Rf values TLC with reversed fase

Compound	[a]	II	VI	V	XIX	III	VII	XII	XX
Mean Rf value	1.00	0.82	0.70	0.65	0.20	0.72	0.61	0.50	0.12
Standard deviation	—	0.058	0.062	0.061	0.048	0.040	0.067	0.062	0.030
Number of determinations	—	14	17	13	17	17	17	14	13

[a] 1-phenylcyclohexanol.

The two tert.butyl compounds do again deviate from the normal course; they show by far the highest lipid solubility.

The Rf value proves to be strongly affected by substitution in both nuclei. If the Rf values are plotted versus the total number of carbon atoms in both substituents together, an almost linear relationship is obtained for all but the tert.butyl compounds (see fig. 14): the Rf value of 1-phenylcyclohexanol ($= 1$) is also situated on this line.

The Rf values of neither of the tert.butyl compounds conform to this relationship.

Although, judging from these results, toxicity apparently is not determined by lipid solubility [b]), we should like to point out once again the deviating behaviour of the two tert.butyl compounds (XIX, XX).

Thin-layer chromatography technique

The method used is that of Boyce and Bilberow.[26] The eluent is a mixture of acetone and water (6:4), which is saturated with the stationary phase.

The best detection is obtained with Ehrlich's reagent ($= 1$ % para-dimethylaminobenzaldehyde in ethanol 96 %).

[b]) In view of the variations between the two series it seems improbable that the sudden increase of the LD_{50} should be a simple result in both series, of the optimal value — for maximal activity — for the lipid solubility being passed, as indicated by Hansch and Fujita[51].

After spraying with this reagent the chromatograms are placed for 5 minutes in a space which is saturated with HCl. The spots take either a violet or a brown colour.
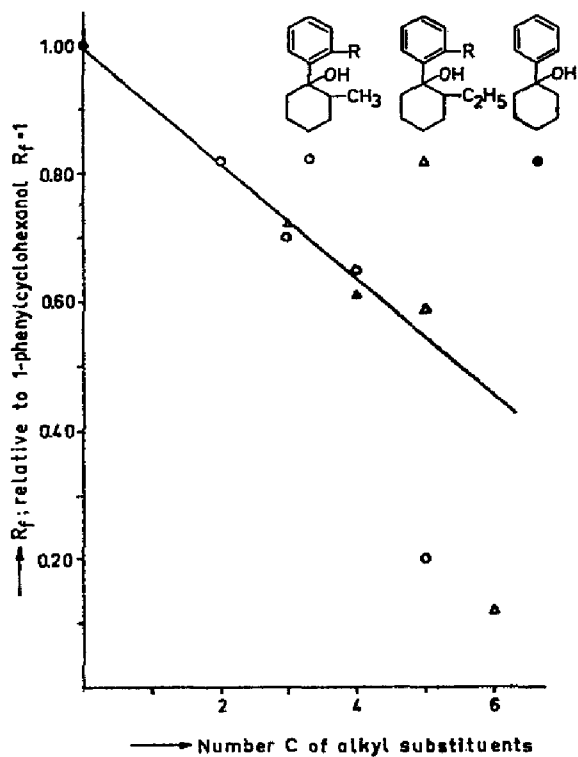

fig. 14 Relationship between Rf values and number of carbon atoms in the side chains.

CHAPTER 3   THE CORONARY PERFUSION OF THE
ISOLATED GUINEA PIG HEART
ACCORDING TO LANGENDORFF

3.1 INTRODUCTION

The mechanism of coronary circulation is a very complex one, and
is influenced in multiple ways. It is as yet not completely clear in
what way the energy need of the heart is adapted to the energy
supply as provided by the blood. However, the lumen of the coronary
vessels logically is a factor of major importance. When all other
factors remain constant increase of the coronary vessel lumen will
lead to an enhanced supply of blood to the myocardium.

Seen purely schematically, one may say, that the coronary circulation
is regulated neurogenically, is dependent of the pressure in the aorta,
and of the heart rate, that it is most certain also influenced by
metabolic processes in the heart muscle, as well as in direct action
on the vascular musculature. Finally, the extravascular pressure due
to the myocardium is of major significance.[23]

Drugs may interfere in each of the above mentioned mechanisms.[66]

The influence as a whole of any drug on the coronary circulation
can be determined only on the intact animal: all regulation
mechanisms will then be able to manifest themselves.

The direct effect on the coronary vessels is best demonstrated on
the isolated heart. The method we used was introduced by
Langendorff.[70] It simplies the insertion of a cannula into the aorta,
– care being taken that the aortic valves are not damaged –, through
which the liquid medium enters. After it has perfused the coronary
vessels it is collected, and measured quantitatively. In this technique
temperature effects are eliminated by working at constant temperature;
the pressure too, is kept at a constant level, but suffers some
fluctuations due to the strength of the heart contractions which are
in their turn, possibly affected by the drug.

The contractions furthermore affect the extra-vascular pressure:
when they become stronger, perfusion, as a result of growing
resistance, will slow down (the phenomenon is possibly somewhat
compensated by the increased pressure): when they become weaker,
resistance lessens, and the perfusion will increase.

62

A considerable difference exists between the effect of the heart rate on the coronary flow in, respectively, the isolated organ, and the intact animal.

Wegria propounds: "– in the whole animal, the effect which a change in heart rate must have on the coronary flow is compensated for, or offset by the other cardiovascular effects of the drug or the agent responsible for the change in heart rate", and for the isolated organ, "– a rise in heart rate per se tends to reduce the coronary flow since it increases the period of lower coronary flow (systole), and decreases the period of higher coronary flow (diastole)."[99] Although the Langendorff technique does not provide information on the over-all action of the drug on the coronary flow, it yielded satisfactory data on how the coronary flow is affected by variations of the lumen of the coronary vessels.[23, 101]

3.2 THE EXPERIMENT ACCORDING TO LANGENDORFF

Use was made of the apparatus as depicted in fig. 15. A guinea pig (average weight 300-400 g), was killed by a blow to the head; we preferred not to remove the heart under anesthesia, as we wished to prevent every possible influence on the coronary flow by the anesthetic. The pleural cavity is cut open and the pericardium opened. In the aorta, which is dissected free, a small incision is made, through which a cannula is introduced filled with perfusion fluid.

The cannula is connected to a small rubber tube, which is closed with a clamp.

The cannula is fastened to the vessel with suturing silk; permanent care is taken not to damage the valves.

The heart is then cut free and removed to a bowl containing perfusion fluid. A short length of suturing silk is drawn through the apex of the left chamber and tied fast. The heart is subsequently connected – via the rubber tube of the cannula – to the perfusion apparatus (at C).

The valve K is now opened to start perfusion of the heart. The thread attached to the left chamber provides registration of the contractions on a smoked drum.

The perfusion liquid used is the solution described by Meyler; repeated checks showed that the pH of this solution remained between
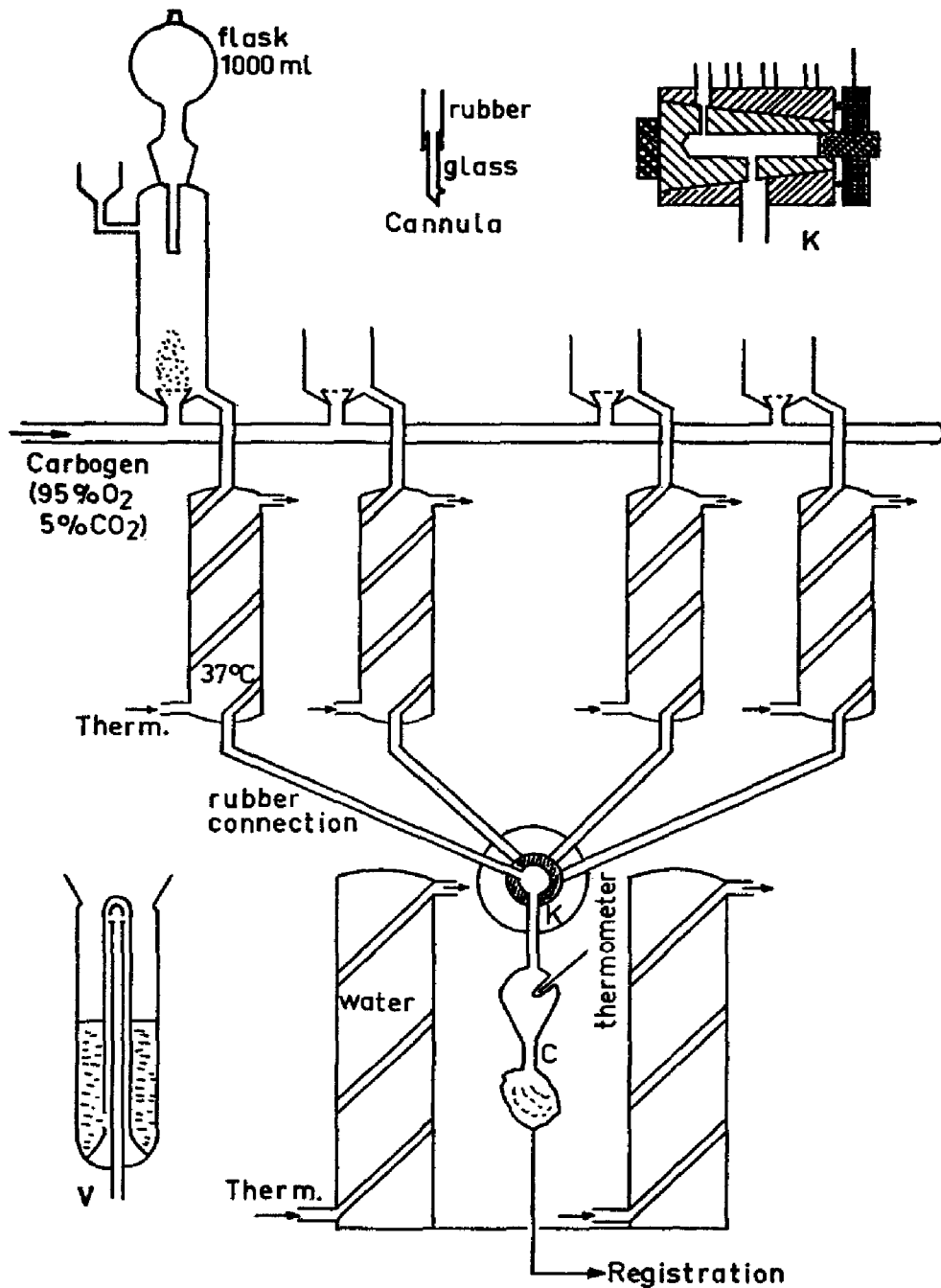
fig. 15 Diagram of the apparatus used for the perfused isolated guinea pig heart (not on scale).

7.3–7.4.[74] This solution will be referred to in the following as Meyler's solution.

The liquid flowing from the heart is collected in a container V (fig. 15) which empties itself itself automatically by means of a siphon. The rate of emptying is noted on the same paper on which

the heart contractions are also registered. The mechanogram thus obtained therefore reveals at the same time the coronary flow, the heart rate, and the amplitude of the heart contraction.

The valve K was constructed so as to allow a quick change to another solution (fig. 15).

The height of the water column – pressure – is throughout maintained at 70 cm. The temperature was 37°C.

The investigation involved the eight compounds mentioned on page 56; viz. the methylcyclohexanols; II, VI, X, and XIX, and the 2-ethylcyclohexanols: III, VII, XI, and XX.

The compounds are all very poorly soluble in water; saturated solutions invariably contain less than 0.1 mg/ml water. The substances were therefore dissolved in propylene glycol, in such a way that for the desired concentration in Meyler's solution always 0.2 ml propylene glycol/1000 ml was used. The influence of propylene glycol itself has been investigated in a separate investigation.

The method used was the following. After the amplitude of the heart contractions and of the coronary flow have become stable we measured the heart rate. Following this, perfusion with Meyler's solution is replaced by perfusion with the same solution to which 0.2 ml propylene glycol/l, and a certain amount of the investigated compound have been added.

Five minutes later we again measured the heart rate, after which, with the exception of a few cases which comprised further experiments with the same heart, the experiment was terminated.

The compounds were found to exert the following effect:

Heart rate – suffers hardly any influence, except for some cases where an exceedingly high concentration, viz. 10^{-4} molar, was used.

Amplitude heart contraction : Very minor effect – slight increase – at concentrations of 10^{-5} molar. At increasing concentrations the amplitude decreases regularly.

Coronary flow – a distinct increase of the coronary flow is seen, both in cases where the amplitude of the heart contraction is still largely unaffected (decrease of the extravascular resistance), as in cases where this amplitude has increased.

Some mechanograms have been depicted in fig. 16.

In addition to the outstanding effect of the compounds on the coronary flow we established also that the compounds could easily be washed out.

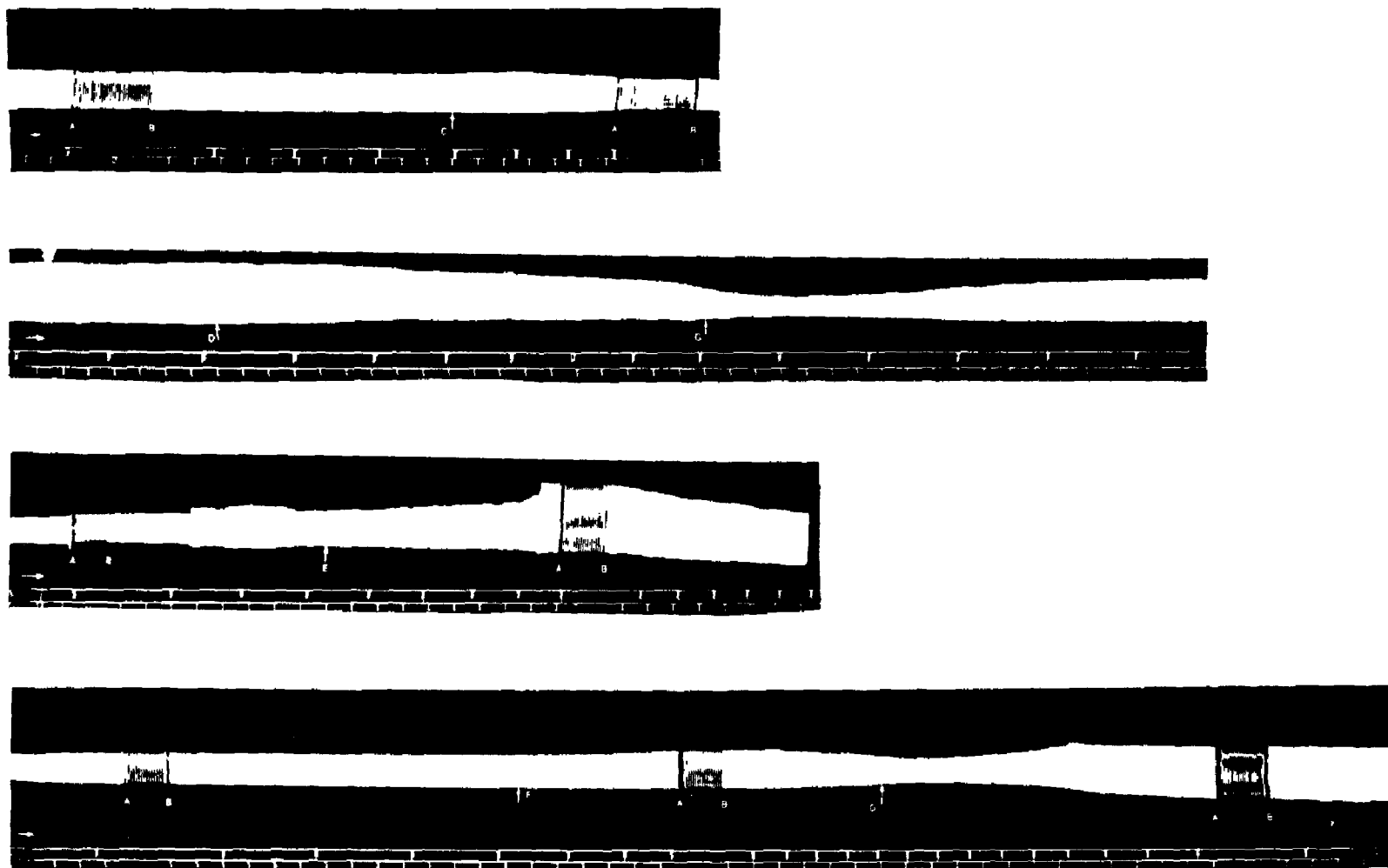fig. 16 Isolated guinea pig heart perfused by means of the apparatus of fig. 15.

From top to bottom: heart contraction, coronary flow (each interval: 24 ml) and time in minutes.

A-B: speed (paper) 4 mm pro sec., to obtain heart frequency. Normal speed 15 mm pro min.

C: II, $c = 10^{-4.5}$ is added.

D: X, $c = 10^{-5}$,, ,,

E: XX, $c = 10^{-5}$,, ,,

F: III, $c = 10^{-5}$,, ,,

G: Perfusion without test compound.
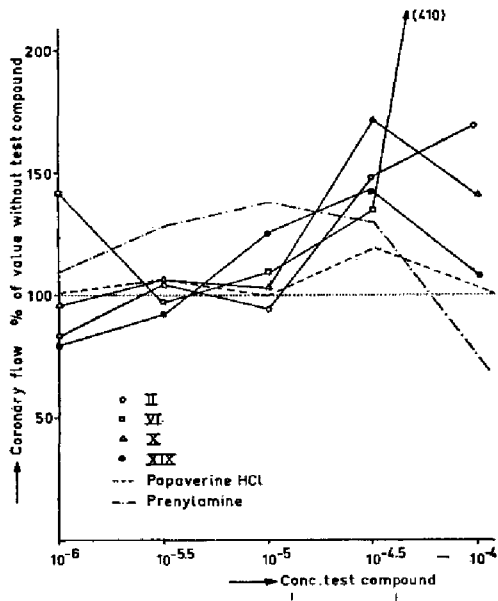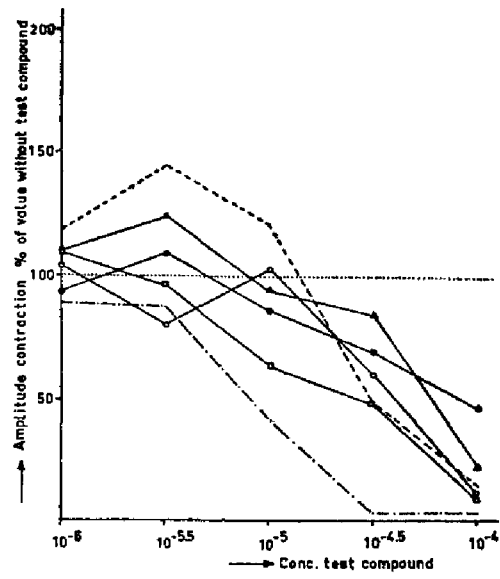
fig. 17 Relation concentration and coronary flow (A); concentration and amplitude heart contraction (B).

fig. 18 Relation concentration and coronary flow (A); concentration and amplitude heart contraction (B).

Some known drugs were investigated in the same way, viz. papaverine hydrochloride and prenylamine [a] (=1.1-diphenyl-N-3'-phenylpropyl-2')-3-aminopropane); both compounds possesses good (coronary) vasodilatory properties in our experiments. In case of prenylamine which is 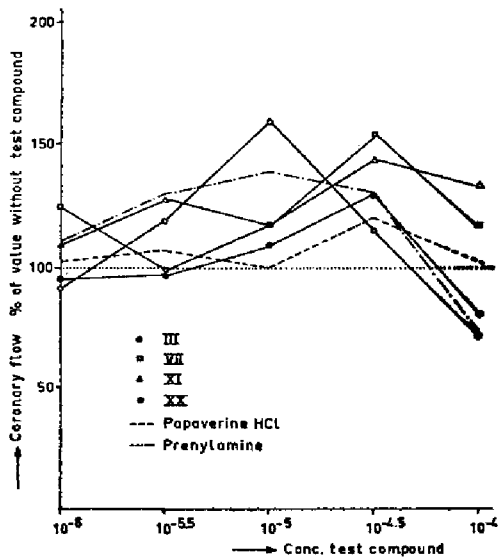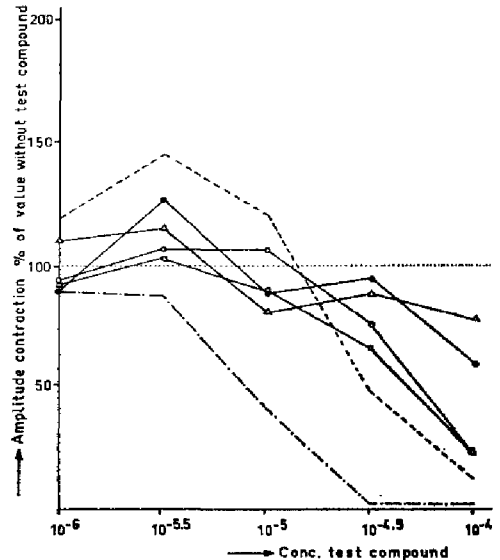still active in a $10\times$ lower concentration a considerable decrease of the amplitude of the heart contraction was a striking feature.

In fig. 17 to 18, the relation between concentration and respectively coronary flow and amplitude of the heart contraction has been depicted graphically. However, one has to make allowance for the influence of 0.2 ml propylene glycol/l of perfusion liquid.

The effect of the substance has been measured after a 5 minutes' perfusion with medium plus investigated substance. This effect has been expressed in per cent of the corresponding values as they are 5 minutes prior to administration.

The influence of the propylene glycol has been measured on 10 hearts on various days. Its influence on the coronary flow, expressed as effect of 5 minutes' perfusion with – in per cents of the flow 5 minutes prior to administration of propylene glycol, with standard deviation, is 86.2 ± 11.8, and for the amplitude of the contraction 91.8 ± 24.8. As is shown in the figs. 17-18, the 2-ethylcyclohexanols prove to exert the stronger effect of the two series of compounds on the coronary flow, while they do not reduce the amplitude of the contraction, even at higher concentrations [b]; prenylamine in low concentration causes a considerable reduction of the amplitude. There are no other apparent relations, however, between structure and activity of the substances.

3.3 EXPERIMENT ACCORDING TO LANGENDORFF, IN WHICH THE CORONARY VESSELS HAVE BEEN CONSTRICTED BEFOREHAND

It was clearly seen from the separate experiments that hearts, of which the coronary flow was initially small, showed percentagewise the greatest increase.

---

[a] segontine®; (Hoechst).
[b] This observation may be connected to LD_{50} values.

Most of our hearts had a coronary flow between 10-20 ml/minute, with extremes of 3.8 and 30 ml/min.

To offset the disadvantage that in the case of a high initial flow the effect of "treatment" is only slight, experiments were carried out in which a vasoconstrictor was added to the perfusion liquid as suggested by Cameron and Craver.[16] The idea expounded by Charlier, to vary the height of the watercolumn ($\equiv$ pressure) for each heart, has not been followed, in order to maintain identical external circumstances for each heart.[23] The method used was the following.

When the heart is beating normally in the apparatus, the perfusion liquid is replaced by the same liquid plus – per liter – 0.2 ml propylene glycol, and 0.1 ml of a solution which contains 10 i.u. extract of the posterior lobe of the pituitary per ml [b].

The perfusion then decreases to about 50 % of the original value, but the amplitude of the heart contractions is not notably affected.

When the coronary flow has become constant, we change over to perfusion with Meyler's liquid, 0.1 ml of posterior lobe extract (see above), and the substance to be investigated, dissolved in 0.2 ml propylene glycol.

The compounds now show a distinctly positive inotropic activity, far superior to that in the experiments without the pituitary extract. The extract seems to heighten the sensitivity of the heart muscle to our compounds, or vice-versa.

We now measure the maximal effect on the coronary flow as compared with the flow measured with the perfusion liquid in which the investigated substance is still lacking.

The results have been depicted percentagewise in fig. 20-21, together with the corresponding values for the contraction amplitude. As was the case for the previous experiments, here too, papaverine and prenylamine were investigated under identical circumstances. We furthermore included 1-phenylcyclohexanol in the investigation. Fig. 20-21 only mentions the results of those experiments in which the investigated compound did not reduce the amplitudes of the contractions to values below 50 % of the original figures.

Again, the investigated compounds started to show activity at concentrations that were 10 times higher thane th lowest active prenylamine concentration.

---

[b] Piton (Organon).

69
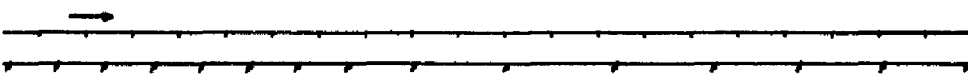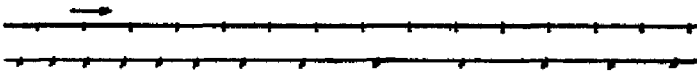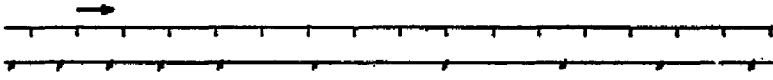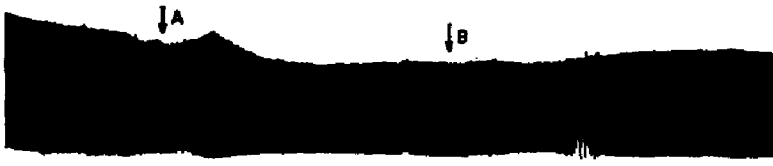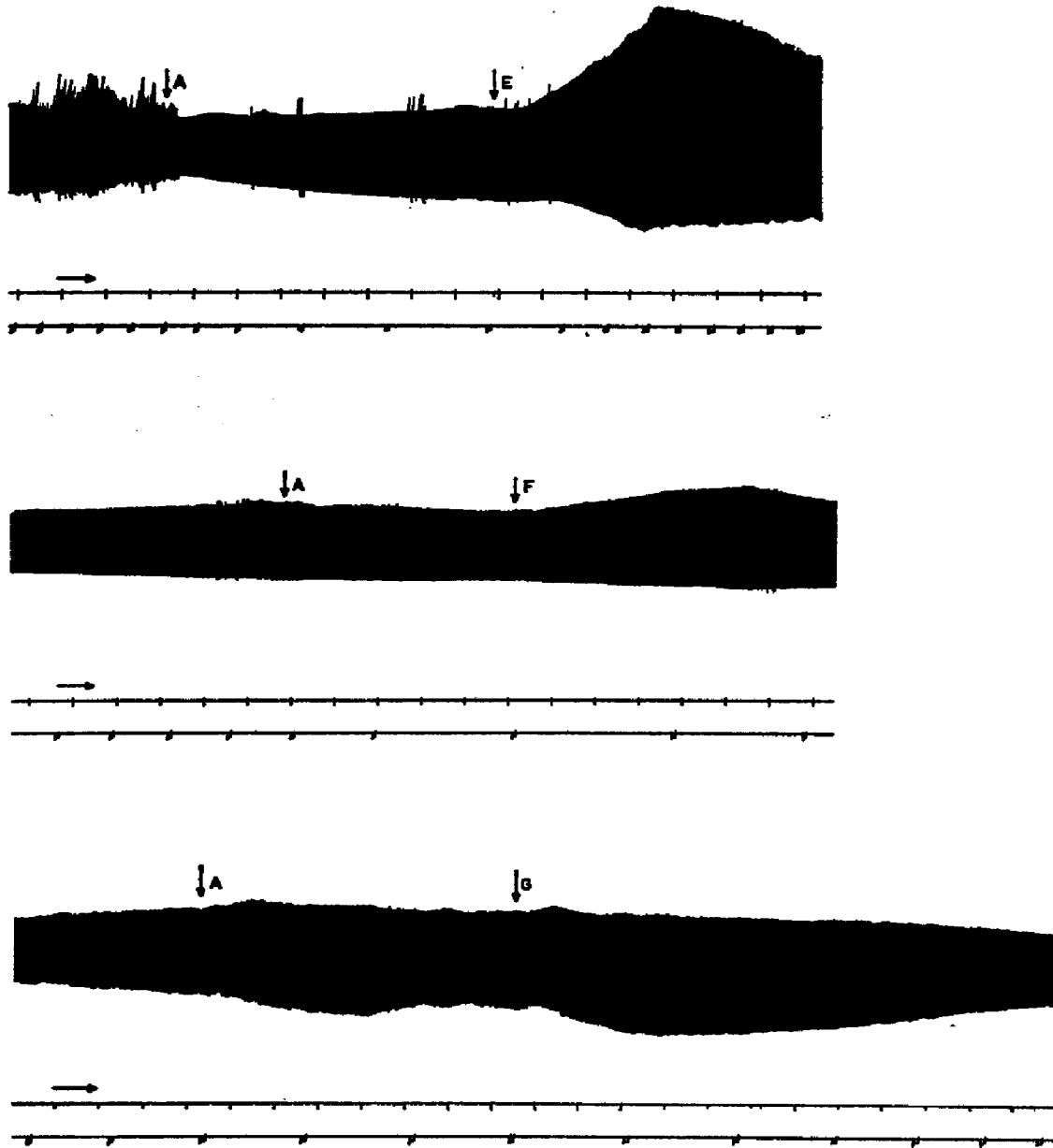
fig. 19 Isolated guinea pig heart perfused by means of the apparatus shown in fig. 15.

From top to bottom, heart contraction, coronary flow (each interval: 18 ml). Speed paper 15 mm/min.

A Pituitary extract is added.

B X, $c = 10^{-5}$, is added   E VII, $c = 10^{-5}$ is added

C XIX, $c = 10^{-6}$, „   „   F VII, $c = 10^{-6}$ „   „

D III, $c = 10^{-6}$, „   „   G XI, $c = 10^{-5}$ „   „

A

B
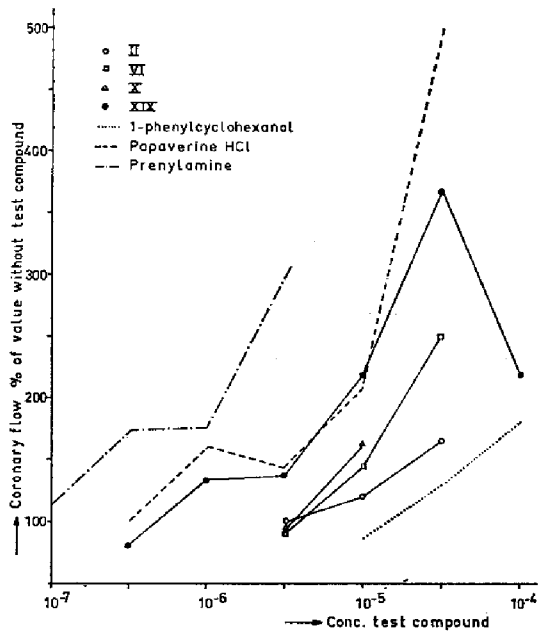
fig. 20A   Relation concentration and coronary flow in hearts with constricted vessels.
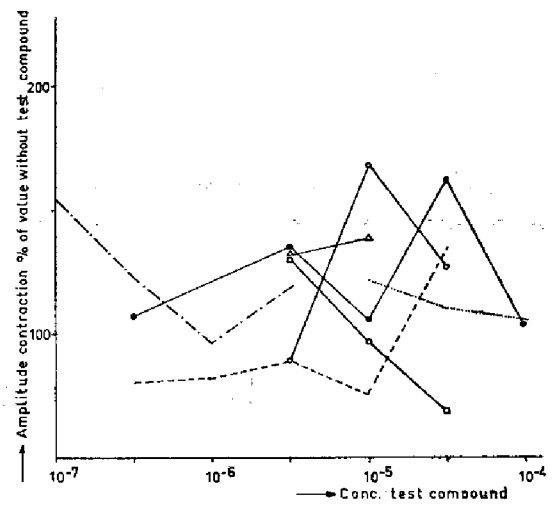
fig. 20B   Relation concentration and amplitude heart contraction (constricted vessels).

A

B
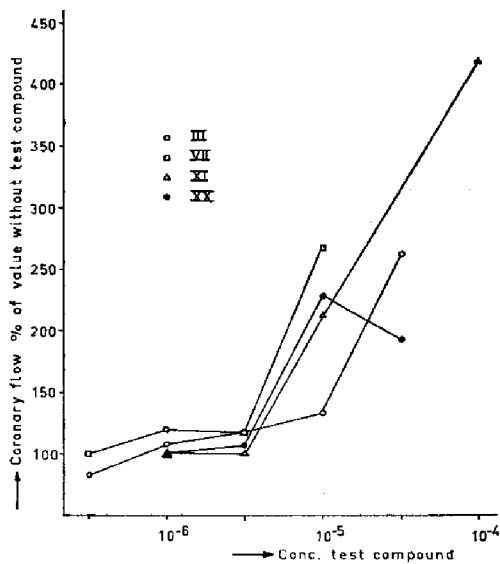
fig. 21A   Relation concentration and coronary flow in hearts with constricted vessels.
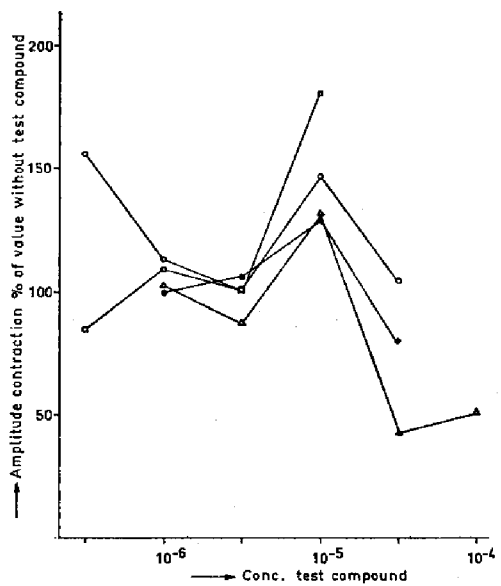
fig. 21B   Relation concentration and amplitude heart contraction (constricted vessels).

72

In the series of methylcyclohexanols the ortho-tert.butylphenyl derivative has a positive action both on the coronary flow as well as on the amplitude of the heart contraction; this activity does extend over a relatively wide range of concentrations. Its analogue in the 2-ethylcyclohexanols does not distinguish itself in the same way.

The toxicity – as the negative influence on the amplitude of the contraction in this experiment – of the 2-methylcyclohexanol compounds increases with increasing bulk of the ortho substituent in the phenyl nucleus up to the propyl derivative. The tert.butyl derivative with the deviating conformation, however, is the least toxic of the series. In the corresponding series of 2-ethylcyclohexanols, the conformation change is not demonstrated so distinctively, although the ortho tert.butylderivative here too, cannot be labelled as toxic.

About the mechanism of the effect on the isolated heart according to Langendorff, little can be said.

In view of the theories quoted in the introduction of this chapter, the increase of the coronary flow can only have been caused by an increase of the lumen of the coronary vessels.

The results of the experiments with constricted vessels – brought about by the pituitary extract – the amplitude of the heart contraction increases under the influence of this extract plus investigated substance – incited us to determine whether the sensivity of the heart muscle does possibly change under the influence of 2-alkyl-1-(ortho-alkylphenyl)cyclohexanols, the results of this investigation are reported in chapter 5.

CHAPTER 4 BLOOD PRESSURE MEASUREMENTS

4.1 DIRECT MEASUREMENT IN ANESTHESIZED RATS [a] AND CATS

Rats of either sex were anesthesized by injection i.p., with urethane (1200 mg/kg).

Blood pressure was measured by means of a mercury manometer, connected to a cannula containing physiological saline and introduced into the carotid artery.

Since all our compounds are very poorly soluble in water, intravenous administration was not very well possible: i.v. injection of up to 10 ml/kg of a saturated solution in saline does not show any effect. The substances were therefore administered intraperitoneally, dissolved in propylene glycol. Doses of up to 1000 mg/kg left the blood pressure unaffected. The same negative result was obtained on replacement of the solvent by dimethylsulfoxide.

In a similar experiment cats were used, who had been anesthesized after an ether induction by means of chloralose, 60 mg/kg.

Following intraperitoneal injection of solutions in DMSO the blood pressure did not change perceptibly.

4.2 INDIRECT MEASUREMENT IN HYPERTENSIVE RATS

Rats with a permanent high blood pressure [b] were obtained according to the method as originally described by Goldblatt et al.; this includes occlusion of the left renal artery with a silver clipping.[10] Some weeks following this the rats have developed a permanent high pressure. The investigation included only rats with a systolic blood pressure above 180 mm Hg.

The blood pressure and the heart rate are measured according to the principle of Riva-Rocci, by means of an Infraton pulse detector (Brecht and Boucke) attached to the caudal artery.[13, 14]

---

[a]) Wistar, SPF 250-300 g.

[b]) Wistar, SPF, male, 180-200 g.

74

The pulse detector is connected to a recorder (ECG apparatus, Schwarzer) which affords the systolic pressure in a simple and reproducible way.

Breuninger does indicate a method to measure also the diastolic pressure, but the values obtained are not sufficiently accurate.[14] When the rats have reached the desired hypertension, blood pressure and heart rate are determined one day prior to administration of the substance to be investigated. These values are taken as the initial values.

Following administration, blood pressure and heart rate are determined after 1, 2, 4, and 8 hours intervals.

Measurements are always performed three times consecutively and the values recorded are the mean of these three.

The compounds were dissolved in cotton seed oil and administered i.p. in 5 and sometimes 10 ml/kg. The influence of cotton seed oil on blood pressure and the heart rate was determined separately.

For each dosage measurements were taken on three rats, included in the investigation on various days. For the cotton seed oil measurements we used eight rats.

Each substance was tested in doses of 250 and 500 mg/kg, while in several cases we also injected doses of 62.5, 125 and/or 1000 mg/kg. Some of the results have been depicted in fig. 22, 24.

A transient drop in blood pressure is noticeable, which in several cases is of considerable extent (fig. 22, 24).

Figure 22 also shows the effect of hydralazine hydrochloride [a]; this preparate was administered orally, suspended in amylum.[5]

There is no distinct relation between dose and extent of lowering of blood pressure. The fall is in some cases more pronounced with doses of 250 mg/kg and in others with 500 mg/kg. Doses of 1000 mg/kg provoked a marked fall, but at this dosage level the condition of the animals was poor.

Fig. 22, 24 merely presents the effect of i.p. injections of 250 mg/kg. The heart rate is also found to be altered by the investigated compounds. However, we might have expected the heart rate to increase as the blood pressure falls, a phenomenon which may be very clearly seen in the case of hydralazine hydrochloride. In stead, the heart rate is seen to decrease. This effect has been illustrated in

---

[a] Apresoline®, 1-hydrazinophtalazine-HCl (Ciba).
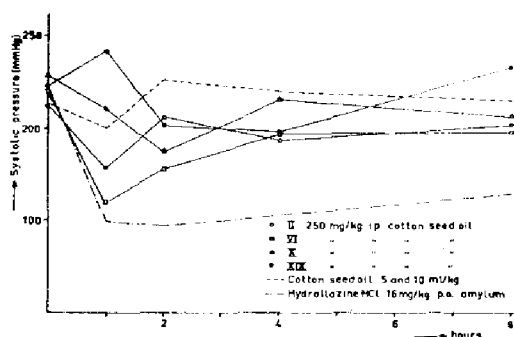
fig. 22 Effect on blood pressure in hypertensive rats of compounds II, VI, X, XIX, cotton-seed oil and hydrazaline hydrochloride.
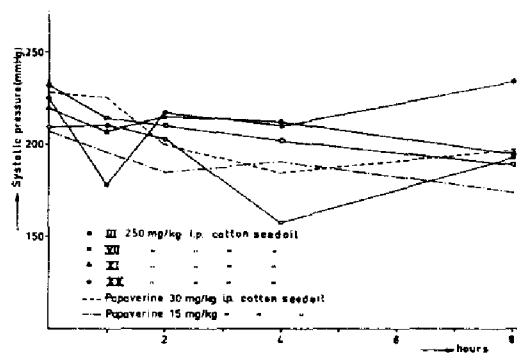
fig. 23 Effect on blood pressure in hypertensive rats of compounds III, VII, XI, XX and papaverine.
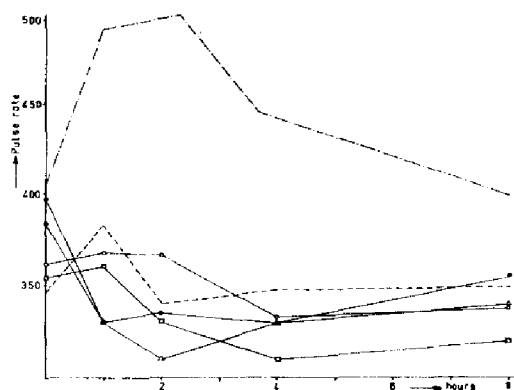
fig. 24 Effect on heart rate in hypertensive rats of compounds II, VI, X, XIX, cotton-seed oil and hydrazaline hydrochloride.
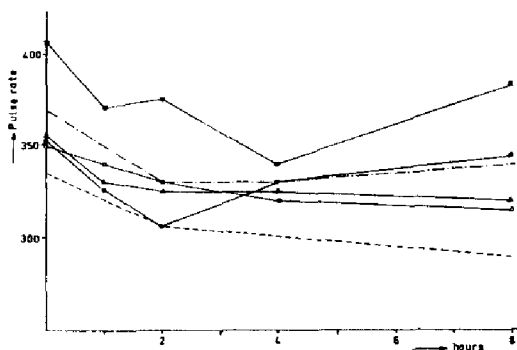
fig. 25 Effect on heart rate in hypertensive rats of compounds III, VII, XI, XX and papaverine.

fig. 23, 25. The decrease in frequency does not appear to be synchronic with the blood pressure fall (see e.g. II, III, XX).

The effects noted are most probably due to the papaverine like action of the compounds. Tested as $BaCl_2$ – antagonists, on the guinea pig ileum according to Magnus, the substances proved 0.5–1 x as active as papaverine-HCl.[72]

Papaverine however, is described in the literature as having not only a lowering effect on the blood pressure, but also as causing tachycardia, after i.v. injection to dogs in doses of 1–1,5 mg/kg.[23,99]

We therefore investigated papaverine in the same way as our own compounds. Since its solubility in cotton-seed oil is very poor,

76

papaverine hydrochloride was dissolved in physiological saline and administered i.p. The LD_{50} of this solution is $\pm$ 50 mg/kg i.p. Fig. 23, 25 show the results obtained with doses of 15 and 30 mg/kg. In renally hypertensive rats papaverine proves to have a similar effect as our own compounds.

It would seem possible that the hypertension caused by occluding the kidney is not comparable to other forms of hypertension.

A recently published series of articles reports that in clinical tests with 2-(2,6-dichloro)phenylamine-2-imidazoline-hydrochloride, administration of this drug to renal hypertensive patients caused a blood pressure fall accompanied by a decreased heart rate.[68]

An equal dose of papaverine does indeed cause a blood pressure fall in normal rats, but a decrease in heart rate cannot be observed; tachycardia is however equally absent.

The cause of the hypertension which results from occlusion of the renal artery is still a subject of controversion.[100]

In the case of renal hypertensive persons a blood pressure fall should be obtainable by influencing the mechanism which causes the increased pressure, thereby normalising the situation. In view of the differences in action of papaverine on, respectively, hypertensive and normal rats, it seems probable that papaverine and papaverine-like compounds act by such a process in the case of renal hypertensive animals. Since the activity of the heart is presumably closely related to the hypertensive condition caused by occlusion of the renal artery, bradycardia may well occur in these cases only.[100]

4.3 INVESTIGATION INTO POTENTIAL ACTIVITY BY WAY OF THE β-ADRENERGIC RECEPTORS

The activity of the compounds could possibly be partially mediated by the β-adrenergic receptors; we hence carried out an investigation into a possible β-sympathicomimetic activity of our compounds.

The method used is that of Waelen, which involves the muscle of the calf's trachea.[98] A contraction is induced with mecholine [a]), which is next abolished by cumulative doses of a β-mimetic, in our case isoprenaline [b]).

---

[a]) mecholine $=$ acetyl-β-methylcholine.
[b]) isoprenaline $=$ ($\pm$)-1-(3.4-dihydroxyphenyl)-2-(isopropylamino)ethanol.

77

In none of the cases the isoprenaline spasmolysis was found to be affected by our compounds. We did note a spasmolysis by our compounds at fairly high concentrations (10^{-4} a 10^{-5}), but papaverine causes a similar effect at a concentration of 10^{-5}, and the above mentioned activity may therefore be ascribed to the papaverine-like properties of the compounds.

THE EXCITABILITY OF THE ISOLATED
PAPILLARY MUSCLE OF THE CAT

4.1 EXECUTION OF THE EXPERIMENTS

The Langendorff experiments revealed that the cyclohexanols do not only affect the perfusion of the coronary vessels, but also the amplitude of the heart contraction. This might indicate a change of the excitability of the muscle. We therefore investigated compounds VI, XI, and XXI, to determine their effect on the strength-duration curve of the papillary muscle of the cat.

The method used was that described by Di-Palma and Mascatello; we preferred however to use the solution according Meyler (Chapter III).[36, 35]

The heart is removed under ether narcosis. The papillary muscles are cut out from the right half of the heart and put in a vessel filled with Meyler's solution: the liquid has a temperature of 30° and a stream of "carbogen" (95 % O_2 and 5 % CO_2) is passed through.

The apparatus used has been partially illustrated in fig. 26. The stimulator (van Gogh) was checked for accuracy of the values as indicated by the dials for frequency, current strength, and duration of the impulse. We used a constant frequency of 60 impulses/minute, with a constant amperage. A papillary muscle is attached to electrode P, in such a way that the tube enclosing the electrode is not completely blocked, but is still able to let some carbogen pass (see fig. 26).

The upper part of the muscle is connected to a writer. The inside of the vessel (A) contains Meyler's solution containing 0.2 ml propylene glycol/l; the temperature is 30°. After the 'warming up' of the muscle (di-Palma and Mascatello) the strength-duration curve is recorded; times of stimulation used are 0.3, 0.6, 1, 2, 3.5, 6, 10 and 15 msec.

We take as the threshold value for a certain impulse duration, the amperage which causes the muscle to contract for at least five consecutive stimulations. The measurements are repeated at 10 minutes' intervals to determine the stability of the values found.

After the strength-duration values have become constant A is emptied through B. Meyler's solution plus 0.2 ml propylene glycol/l

(containing the compound to be investigated in the desired concentration) is then introduced by way of EC.

The preparation is washed three times with this solution which has been warmed to 30° beforehand. After 10 minutes the strength-duration curve is again measured.

For each concentration we recorded 5 curves, on muscles of different cats; each compound was investigated at three different concentrations. The mean of these 5 curves is given.[12] In addition to compounds VI, XI, and XX, we also included bamethan sulphate [a] in the investigation.

This compound does exert, as do compounds VI, XI, and XX to a markedly lesser degree, a positive inotropic effect on the heart muscle in the Langendorff experiment, but its influence on the coronary flow is much less pronounced (see table 16). A frequency increase may be noted (11.5 %, at a concentration of 10^{-5}, 3 hearts).

TABLE 16

Relation $\dfrac{\text{coronary flow}}{\text{amplitude heart contraction}}$ and concentration of bamethan sulphate (Langendorff)

Concentration	10^{-3}	$10^{-3.5}$	10^{-4}	10^{-5}	10^{-6}
Change in amplitude [b]	21	300	156	210	98
Change in coronary flow [b]	83	106	118	92	92

[b] Percents; five min. after administration bamethan sulphate to five min. before administration.

4.2 RESULTS AND DISCUSSION

In fig. 27 several strength-duration curves have been depicted (average of five). The remaining results are given in fig. 28. This also shows the rheobase change as dependent from the concentration. (The rheobase is the minimal stimulation strength which can make the muscle contract; prolongation of the stimulation below this

[a] 2-butylamino-1-p-hydroxyphenyl-ethanol-sulphate; Vasculat® (Boehringer-Ingelheim).

80

minimal strength, does not result in contraction.) Bamethan sulphate and compound XX increase the excitability in all cases; the rheobase, in the case of VI and XI, proves decreased at the two lowest concentrations, but there is a sudden reversal at the highest concentration: the rheobase is increased considerably (decreased excitability).
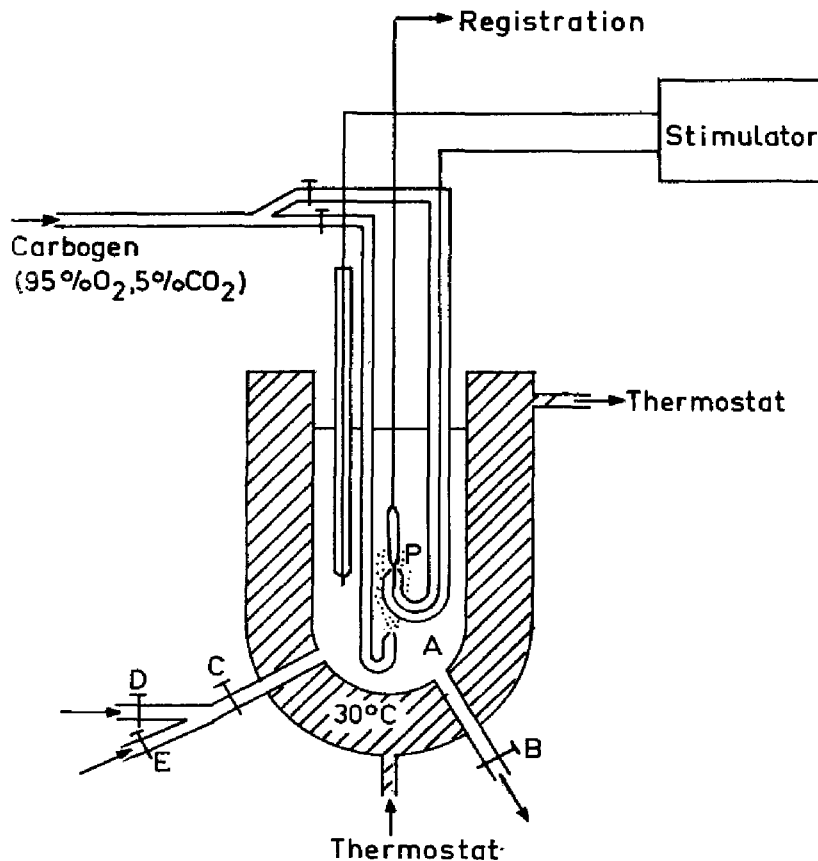
fig. 26 Diagram of the apparatus used for determining excitability of cats papillary muscle.

The time factor for the excitability, the chronaxy, which is the time corresponding to a stimulation strength of twice the rheobase value, remains almost unaltered in all our experiments. The sudden decrease in excitability seen at high concentration is most probably already a result of toxic affection on the muscle.

The above experiment does not afford insight in the mechanism by which the excitability increases. We can but point out the correlation found with the results of the Langendorff experiment. Although the excitability of the muscles seems not to be directly coupled to

the amplitude of the mechanical contraction, nor to the automaticity in the case of the heart muscle, we consider ourselves justified when concluding from the experiments with the constricted heart to an increased excitability of the heart muscle.[85, 96]

Incidentally, acetylcholine proves to increase the rheobase however, therefore decreasing the excitability in the isolated papillary muscle
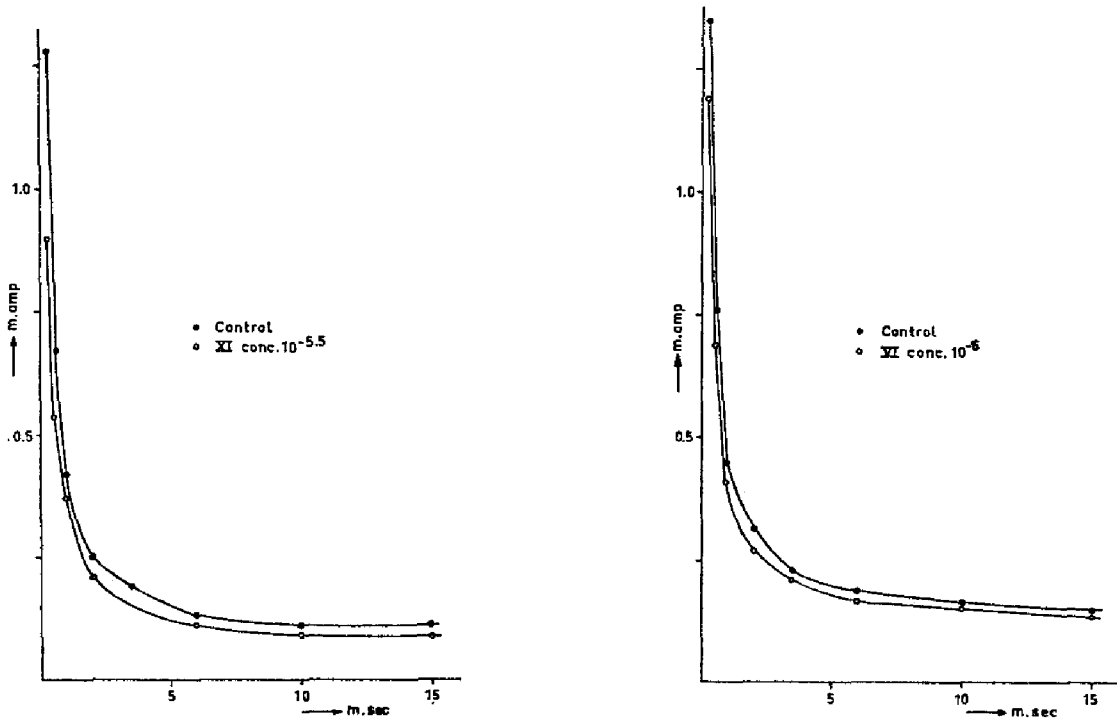
fig. 27   Strength duration curves of papillary muscle of the cat, before and 10 min. after addition of test compounds.
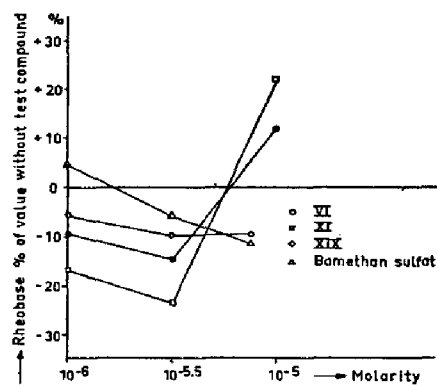
fig. 28   Correlation concentration and change in rheobase; strength duration curves.

of the cat and the auricle of the cat, while decreasing the amplitude of the isolated atrium of the guinea pig.[36, 47]

In contrast, adrenaline caused rather an increase of the excitability, and an increased amplitude in the same experiments.[36, 47]

Notwithstanding a possible absence of any direct link between the two phenomena the changes brought about by acetylcholine and by adrenaline run parallel to those brought about by bamethan sulphate, XX, and in low concentrations, VI and XI.

CHAPTER 6 THE METRAZOL THRESHOLD FOR CLONIC CONVULSIONS

Apart from the LD_{50} values the observed biological activities proved hardly structure dependent. In general the activity of the investigated compounds resembles that of papaverine.

The determination of the LD_{50} values — chapter 1 — revealed that the compounds were neurotoxic at high doses; lower doses did not show this effect. High doses induced a condition in the mice, which resembled anesthesia; the animals tolerated being brought in supine position. In the literature hypnotic properties have been described for some cyclohexanols.[93]

The sedatives-hypnotics are known to be extremely effective in suppressing the minimal clonic seizures induced by metrazol [a]), this in contrast to other depressives. In general a substance with hypnotic properties also raises the metrazol threshold.[2, 24]

We investigated the effect of both series on the metrazol threshold for minimal convulsions in mice [b]). The investigated compound was administered invariably beforehand. After a certain interval metrazol is injected i.v. at a constant speed until the first signs of clonic seizures occur. The dose used for the substances was 6.5 mmol/kg; it was chosen so high to ensure the strongest possible effect in order to make the differences between the compounds larger.

The compounds were administered to groups of ten mice each, obtained by randomisation. The substances were dissolved in cotton seed oil, of which 10 ml/kg was always injected intraperitoneally. In addition to the eight afore mentioned compounds the investigation also included cyclohexanol (6.5 mmol/kg).

A group which had only received 10 ml cotton seed oil per kg served as control. The metrazol threshold was determined one hour following administration of the substances.

---

[a]) Metrazol = pentamethylenetetrazol.
[b]) Swiss random bred, S.P.F., ♂

We noted the number of ml of a metrazol solution in physiological saline (2 mg/ml) that had to be injected into a caudal vein to bring about the first clonic seizures.

A special analysis showed a relation between the weight of the mouse and the amount of metrazol, necessary for seizures [a].

We therefore calculated the mean amount of metrazol – ml solution – per group expressed in ml per average weight of all mice (17.0, geometrical mean).

The results have been listed in table 17, which also gives the

$$\text{MED ratio} = \frac{\text{mean MED (test compound)}}{\text{mean MED (control)}}, \text{ with } 95\ \% \text{ confidence}$$

interval.

TABLE 17

Effect on the metrazol threshold

compound	mean amount of metrazol [b]	med ratio, with 95% confidence interval [b]	significance [d]
control [b]	34.6	1.00	~
II	± 1.1 [c]	± 3.1	+
VI	0.591	1.71 (1.56–1.86)	+
X	0.409	1.18 (1.08–1.29)	+
XIX	0.365	1.06 (0.97–1.15)	—
III	0.568	1.64 (1.50–1.79)	+
VII	0.425	1.23 (1.13–1.34)	+
XI	0.346	1.00 (0.92–1.09)	—
XX	0.364	1.05 (0.96–1.17)	—

[b]) See text.
[c]) Through strong influence by II, relatively inaccurate.
[d]) According to covariance analysis.

The most striking of these results is the fact that substitution lowers the activity. Cyclohexanol has an activity which is slightly stronger than that of VI. Unfortunately the toxicity of 1-phenylcyclohexanol is too high to permit its being tested in this way.

A dose-activity curve of the most active compound – II – is given in fig. 29. The dose-activity relation is very obvious. At low concentration 1-phenylcyclohexanol proved slightly more active than compound II.

From the important activity of cyclohexanol, 1-phenylcyclohexanol and II, VI, and III we may presume the hydroxyl group to be important for this property. This view is supported by the slight activity of the 2-ethylcyclohexanols as compared to the 2-methylcyclohexanols: the hydroxyl group has to be "free".
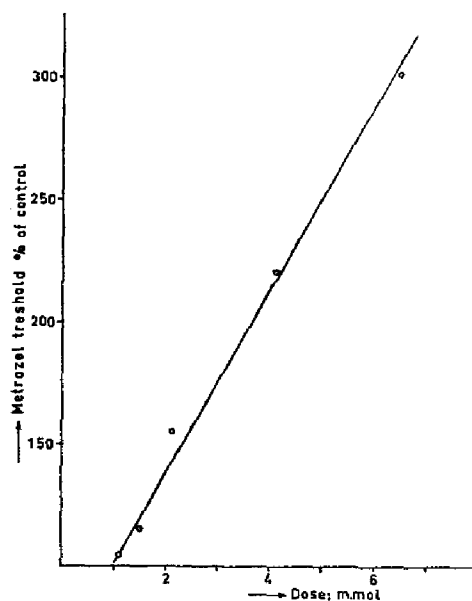
fig. 29 Dose-activity curve of 2-methyl-1-ortho-tolylcyclohexanol on the metrazol threshold.

There is no evident relation with the LD_{50} values; the major difference between the LD_{50} of cyclohexanol and that of 1-phenylcyclohexanol and the high activity of both compounds in the metrazol tests clearly show the lack of any such relation.

In comparison to the other biological activities of our compounds this activity is strongly structure dependent indeed.

The opinion expressed in the introduction of this thesis as to the

chances to recognize the conformation shift as described in the chemical part, in the biological properties of both series is fully justified, however.

The equatorial position of the hydroxyl group in XIX and in XX is not sufficient to guarantee the necessary freedom: the ortho tert.butyl group interferes.

Although the effect is, properly speaking, not significant, a slight influence of XX may be observed (see table 17), while in XI it is completely lacking.

The mechanism by which the metrazol threshold is raised is not known; the fact that our compounds show this activity sure will be connected with the – weak – hypnotic properties.

SUMMARY

Of all the compounds synthesized by us we investigated only the 1-aryl-2-methyl- and the 1-aryl-2-ethylcyclohexanols on their biological properties, since in these two series of compounds the conformation transition in the cyclohexane ring occurs, as described in the chemical part.

The LD_{50} – acute determination in mice after i.p. administration – proved to be strongly structure dependent. The 1-(ortho-tert.butylphenyl)cyclohexanols, which show a deviating conformation compared to the other compounds, are far less toxic than the other three cyclohexanols of the series.

The course of the LD_{50} in either series proved independent from that of the lipid solubility. The lipid solubility as such has not been determined; the Rf values obtained by way of reversed phase thin-layer chromatography do give a fair representation of the relative lipophilic character of the compounds.

In the isolated guinea pig heart according to Langendorff the eight 1-arylcyclohexanols investigated, do show the cardioplegic properties reported in the literature for the 2-tolylcyclohexanols, though only if the substances are present in the perfusion liquid in high concentrations. In the course of this introductory investigation we noted with interest a marked coronary vasodilatory activity.

This activity was then further investigated, using the same preparation. Structure-activity relationships were all but absent; the 2-ethylcyclohexanols had the most favorable effect, inducing the greatest increase of the coronary perfusion without influencing negatively the amplitude of the heart contraction, even at high concentrations.

Hearts, which showed a high initial rate of coronary perfusion were found to be much less affected, both procentually and absolutely, by the test-compounds than hearts with a poorer perfusion; we therefore also investigated hearts constricted by pituitary extract.

The joint effect of the test compounds and the pituitary extract proved to be a distinctly positive inotropic action on the heart-muscle.

Again, the ortho-tert.butylphenyl derivatives stand out, in particular in the series of the 2-methylcyclohexanols.

88

In both experiments papaverine and prenylamine were used as reference substances.

The investigated compounds were shown to have an effect on the coronary perfusion comparable to that of papaverine. Prenylamine has a stronger effect on the perfusion, but in comparison to our compounds, it shows a strongly negative effect on the amplitude of the heart contraction, in much lower concentrations.

No influence on the blood pressure was observed after doses of up to 1000 mg/kg i.p., – due to the insufficient solubility in water i.v. administration was not possible. –

In renal hypertensive rats (Goldblatt), doses of 250 mg/kg (also i.p.) effect a transient fall of the systolic blood pressure. This hypotensive effect is however not accompanied by an increased heart rate. In the case of hydralazine (hydrochloride) the blood pressure fall is much prolonged, but here the heart rate is markedly increased. Although papaverine has been reported to induce in non-anesthesized dogs not only a drop in blood pressure, but also an increase of the heart rate, we were unable to observe the latter affect in renal hypertensive rats. In these animals doses of 15 and 30 mg/kg (of papaverine), i.p., did cause hypotension, but bradycardia was also observed, while in normal rats hypotension but no bradycardia was induced.

The curious effect on the heart rate in renal hypertensive animals has to be ascribed to the abnormal condition of renal hypertension. The investigated compounds increase the amplitude of the heart contraction, in particular in the case of hearts constricted by pituitary extract. Some compounds, viz. VI, XI, and XX were investigated for effect on the excitability of the heart muscle.

For this purpose we recorded strength-duration curves of the isolated papillary muscle of the cat.

Concentration of $< 10^{-5}$ heighten the excitability. Compound XX, 1-(ortho-tert.butylphenyl)-2-ethylcyclohexanol still has this effect at a concentration of 10^{-5}.

Though theoretically there is no imperative for a relation between increased amplitude and heightened excitability, many of the investigated compounds proved to possess both these properties.

The compounds all have a papaverine-like action; tested as $BaCl_2$ antagonists on the isolated guinea pig intestine the activity is 0,5–1 × that of papaverine. The effect of the compounds on coronary perfusion and blood pressure may very well be due to their papaverine-like activity.

When investigated for possible interaction with β-adrenergic receptors, the compounds failed to reveal any such activity.

Only at fairly high concentrations we did note a spasmolysis of the calf's tracheal muscle, which had been induced to contract by means of mecholine, but papaverine in somewhat lower concentrations has the same effect. Moreover such effects are in general little specific. It should cause no surprise therefore, that the activities of the eight compounds are so much alike.

Several cyclohexanols have been reported to be active as hypnotics. Whenever present, such properties might be more specific, and the compounds were hence investigated as to their hypnotic properties. In doses below the toxicity level, hypnotic effects were almost completely absent, however. Since all sedative-hypnotics are known to raise the metrazol threshold we next investigated this aspect, for which we administered our compounds to mice.

The only compounds found to raise the metrazol threshold significantly were those carrying small alkyl substituents: the 2-methylcyclohexanols up to the ortho-cumenyl compound, and in the corresponding ethyl series only the o-tolyl and the o-ethyl-phenyl compound. The ortho-tert.butyl compounds, which carry the hydroxyl equatorially do not raise the metrazol threshold significantly: the conformation transition does therefore not manifest itself here.

Since the effects of the conformation transition within both series – see chemical part – are opposed to one another with regard to the crowding round the hydroxyl group, the distinct correlation between this transition and the acute toxicity – LD_{50} values –, is much more striking than the lack of such a correlation with the other properties.

The pharmacological investigations did reveal some interesting properties. Unfortunately the poor solubility in water prevented administration by the intravenous route, which might have allowed a better demonstration of the effect on smooth muscle (papaverine) in vivo.

Introductory experiments with hydrochlorides of the β-dimethyl-amino-ethyl-ethers of the investigated compounds revealed that they also possess a strong dilatory action on the coronary vessels.

Since these derivatives are freely soluble in water, they might give more accurate results in in vivo tests; the correctness of this supposition will be tested in future experiments.

SAMENVATTING

De conformatie van cyclohexaan derivaten is de laatste jaren het onderwerp van veel onderzoekingen geweest. Algemene regels, die selectiviteit bij addities aan ketonen voorspellen, bleken voor cyclohexanonen niet toe te passen. Voor deze verbindingen werden, onder andere door Dauben, andere regels opgesteld; deze zijn meestal afgeleid voor reductie reacties. De selectiviteit wordt bepaald gedacht door zowel sterische als energetische factoren.

In dit onderzoek wordt aan de hand van literatuurgegevens aangetoond, dat bij reacties tussen cyclische ketonen en organo-metaalverbindingen, als Grignard reagentia en Li-verbindingen, sterische factoren de selectiviteit bepalen. Wanneer het metaalcentrum in het organo-metaal reagens en/of de carbonyl functie in het keton door grotere groepen wordt omgeven, dan neemt de bevoordeling van nadering van de minst gehinderde, dat is bij cyclohexanonen de equatoriale zijde, van de carbonyl-groep door het organo-metaal reagens toe. Equatoriale nadering heeft een axiale plaatsing van de gevormde hydroxyl-groep ten gevolge. Aan de hand van deze gegevens wordt voorspeld, welke de *configuratie* zal zijn van het éne isomer, dat, blijkens gaschromatografische analyses, wordt gevormd bij reactie tussen ortho-alkylphenyllithium-verbindingen en 2-alkyl-cyclohexanonen.

Uit de NMR spectra van de gevormde 2-alkyl-1-(ortho-alkylphenyl)-cyclohexanolen wordt aangetoond, dat de voorspelling, dat het *trans* isomeer gevormd zal zijn, juist blijkt te wezen, in alle gevallen. In de ortho-*tertiaire* alkylphenyl verbindingen evenwel blijkt niet de voor de hand liggende *conformatie* met axiale hydroxyl-groep aanwezig te zijn — de plaats van het signaal van het hydroxyl proton wordt vergeleken met literatuurgegevens; de overige cyclohexanolen bezitten wel deze conformatie. Dat de tert.alkylphenyl derivaten toch de trans produkten zijn, ontstaan door equatoriale nadering, gevolgd door ringinversie, wordt aangetoond door de signalen van de substituenten op de 2-plaats van de verzadigde kern. De ringinversie is het gevolg van een sterke interactie tussen de ortho-tert.alkylgroep en de substituent op de 2-plaats van de cyclohexaanring, die niet optreedt wanneer de ortho substituent primair of secundair is. Dit verschijnsel kan aan modellen gedemonstreerd worden.

In gevallen waar ringinversie onmogelijk is, doordat de cyclohexaan-ring door substituenten in één bepaalde conformatie wordt gedwongen – tert.butyl gesubstitueerde cyclohexyl en trans-decalyl-verbindingen – blijken de ortho-tert.butylphenyl derivaten inderdaad dezelfde conformatie als de overeenkomstige verbindingen zonder tertiaire ortho substituenten te bezitten.

Van de gesynthetiseerde verbindingen zijn slechts de 1-aryl-2-methyl-en 1-aryl-2-ethylcyclohexanolen onderzocht op hun biologische eigenschappen, omdat in deze twee series verbindingen de in het chemische gedeelte beschreven conformatie-overgang van de cyclo-hexaan-ring aanwezig is.

De LD_{50}-waarden – acute bepaling bij muizen, na i.p. toediening – blijkt sterk structuur afhankelijk te zijn. De 1-(ortho-tert.butyl-phenyl)cyclohexanolen welke een ten opzichte van de overige ver-bindingen afwijkende conformatie bezitten, hebben een veel ge-ringere toxiciteit dan de drie andere cyclohexanolen uit de reeks. Het verloop van de LD_{50} in beide reeksen bleek niet te correleren met dat van de vetoplosbaarheid. De vetoplosbaarheid is als zodanig niet bepaald; de Rf-waarden – verkregen bij dunnelaag chromato-grafie met reversed phase – geven evenwel het lipofiele karakter van de verbindingen ten opzichte van elkaar goed weer.

De achter in het onderzoek betrokken 1-arylcyclohexanolen vertonen de bij de 2-tolylcyclohexanolen – blijkens de literatuur – duidelijk aanwezige cardioplegische eigenschappen slechts bij hoge concen-traties van de stoffen in de doorstromingsvloeistof, bij het ge-isoleerde caviahart in de opstelling volgens Langendorff. Bij dit inleidende onderzoek kwam evenwel duidelijk een coronair vasodila-toire werking naar voren.

Deze werkzaamheid is daarna verder onderzocht, in dezelfde opstel-ling. Er is maar weinig verband tussen structuur en werking te ontdekken; de 2-ethylcyclohexanolen blijken het gunstigst te zijn in die zin, dat ze de coronair doorstroming het sterkst vergroten en de amplitude van de hart-contractie tot hogere concentraties niet negatief beinvloeden.

Daar het bleek dat bij harten met initieel grote coronairdoorstroming procentueel en absoluut veel minder invloed op deze grootheid door de test-verbindingen valt waar te nemen dan bij harten met geringere doorstroming, zijn er ook experimenten uitgevoerd met door hypofyse extract geconstringeerde harten. Hierbij bleek dat het samenspel van

92

de testverbindingen en het hypofyse extract een duidelijk positief inotrope werking op de hartspier veroorzaakt. Ook vallen de ortho-tert.butylphenyl derivaten weer op, vnl. in de reeks van 2-methyl-cyclohexanolen.

Bij beide experimenten zijn papaverine en prenylamine als vergelijk-stoffen gebruikt. De verbindingen hebben in dit experiment een werking op de coronairdoorstroming die te vergelijken is met de werking van papaverine. Prenylamine is sterker werkzaam op de coronairdoorstroming, maar blijkt, ten opzichte van de door ons onderzochte verbindingen, in lage concentraties een sterk negatieve invloed te hebben op de amplitude van de hartcontractie.

Invloed op de bloeddruk is na i.p. injectie – vanwege de geringe oplosbaarheid in water is intraveneuze toediening niet mogelijk – niet waargenomen, tot doses van 1000 mg/kg. Renaal hypertensieve ratten (Goldblatt) vertonen na doses van 250 mg/kg – eveneens intraperitoneaal – een vrij kortdurende daling van de systolische bloeddruk. Aan dit hypotensieve effect is echter geen stijging van de frequentie gekoppeld. Bij hydralazine (hydrochloride) is bij de, veel langer durende, bloeddrukdaling wel een sterke stijging van de hartfrequentie geconstateerd. Hoewel de literatuur – voor onge-narcotiseerde honden – aan papaverine naast een bloeddrukverlagende ook een frequentieverhogende werking toeschrijft, blijkt dit voor renaal hypertensieve ratten wat de frequentieverhoging betreft, niet op te gaan. Doses van 15 en 30 mg/kg (papaverine), i.p., veroorzaken wel een hypotensie, maar tevens is bradiecardie waar te nemen. Bij normale ratten treedt naast hypotensie, onder invloed van papaverine, geen bradiecardie op. Het merkwaardige effect op de hartfrequentie bij renaal hypertensieve individuen moet worden toegeschreven aan de niet normale situatie bij renale hypertensie.

De onderzochte verbindingen vergroten de amplitude van de hart-contractie, vooral bij met hypofyse extract geconstringeerde harten. Enkele verbindingen, VI, XI, en XX, zijn onderzocht op de invloed die zij hebben op de prikkelbaarheid van de hartspier.

Tijd-intensiteitscurves werden daartoe opgenomen van de ge-isoleerde papillairspier van de kat. Tot een concentratie $< 10^{-5}$ wordt de prikkelbaarheid vergroot. Verbinding XX, 1-(ortho-tert.butylphenyl)-2-ethylcyclohexanol verhoogt de prikkelbaarheid ook nog bij een concentratie van 10^{-5}. Hoewel er theoretisch geen ver-band behoeft te bestaan tussen vergrote amplitude en verhoogde prik-kelbaarheid blijken veel verbindingen beide eigenschappen te bezitten.

93

De verbindingen hebben alle een papaverine-achtige werking; getest volgens de methode van Magnus als $BaCl_2$ antagonisme op de cavia-darm, is de werkzaamheid 0.5–1 × de werkzaamheid van papaverine. Het is waarschijnlijk dat de werking op coronair-doorstroming en bloeddruk op het papaverine-achtige karakter van de verbindingen kunnen worden teruggevoerd. Bij het onderzoek naar eventuele werkzaamheid op de β-adrenergische receptoren, kon een dergelijke activiteit niet worden aangetoond. Bij relatief hoge concentraties treedt er wel een spasmolyse op van het kalfs-trachea-spiertje dat met mecholine tot contractie is gebracht, maar dat effect veroorzaakt in iets lagere concentraties papaverine ook.

Dergelijke effecten zijn over het algemeen weinig specifiek. Het mag dan ook niet verwonderen, dat er zo weinig verschil in de werkzaamheid van de acht verbindingen is gevonden.

Aan verschillende cyclohexanolen worden ook slaap-verwekkende eigenschappen toegeschreven. Daar het mogelijk was dat deze eigenschap – indien aanwezig – meer specifiek is, zijn de verbindingen op hypnotische eigenschappen onderzocht. Er bleek echter nagenoeg geen slaapverwekkende invloed waar te nemen, beneden toxische doses. Daar alle sedatieve-hypnotica de metrazol-drempel verhogen is daarna de invloed nagegaan die de stoffen hierop hebben, bij muizen.

Het blijkt dat slechts de verbindingen met relatief kleine alkyl-substituenten de metrazol-drempel significant verhogen: in de 2-methylcyclohexanolen tot en met de ortho-cumenyl verbinding; in de overeenkomstige ethyl-serie verhogen alleen de o-tolyl en de o-ethylphenyl verbinding de drempel significant. De ortho-tert.butyl-verbindingen, die de hydroxyl equatoriaal dragen, verhogen de metrazol-drempel niet significant, de conformatie-overgang wordt dus niet gedemonstreerd.

Gezien het feit dat de bij de conformatie-overgang binnen beide reeksen – zie chemische deel – betrokken effecten ten aanzien van de ruimtelijke vulling rond de hydroxyl-groep, aan elkaar tegengesteld zijn, frappeert de duidelijke correlatie van deze overgang met de acute toxiciteit – LD_{50}-waarden – meer, dan de afwezigheid ervan bij de andere eigenschappen.

De farmacologische onderzoekingen brengen enkele interessante eigenschappen aan het licht. Helaas belemmert de geringe oplosbaar-heid in water intra-veneuze toediening, welke mogelijk de werking op de gladde spieren – papaverine – in vivo beter zou doen uitkomen.

94

Inleidende experimenten met zoutzure zouten van de β-dimethyl-aminoethyl-ethers van de onderzochte verbindingen toonden aan dat deze de dilatoire werking op de coronair vaten in sterke mate vertonen. Daar deze derivaten goed in water oplossen zullen in vivo-experimenten hiermee waarschijnlijk duidelijker resultaten opleveren; de juistheid van deze veronderstelling zal in de toekomst worden getoetst.

LIST OF REFERENCES

1	N. L. Allinger, H. Blatter, L. A. Freiberg and F. M. Karbowski,	J. Am. Chem. Soc. 88, 2999 (1966)
2	J. W. Bastian,	Arch. Int. Pharmacodyn. 133, 347 (1961)
3	A. H. Beckett, A. F. Casy, G. Kirk and J. Walker,	J. Pharm. Pharmacol. 9, 939 (1957)
4	A. H. Beckett, N. J. Harper, A. D. J. Balon and T. H. E. Watts,	Tetrahedron 6, 319 (1959)
5	H. J. Bein, F. Gross, J. Tripod and R. Meier,	Schweiz. Med. Wschr. 84, 336 (1953)
6	L. A. Bigelow,	Org. Synth. 9, 22 (1929)
7	A. J. Birch and R. Robinson,	J. Chem. Soc. 1944, 501
8	J. J. Blanksma and O. Hoeg,	Rec. Trav. Chim. 65, 333 (1946)
9	H. H. Bosshard, R. Mory, M. Schmid and Hch. Zollinger,	Helv. Chim. Acta 42, 1653 (1959)
10	F. A. Bovey, F. P. Hood III, E. Pier and H. E. Weaver,	J. Am. Chem. Soc. 87, 2060 (1965)
11	C. B. C. Boyce and B. V. Milborrow,	Nature 208, 537 (1965)
12	O. L. Brady and R. N. Cunningham,	J. Chem. Soc. 1934, 121
13	K. Brecht and H. Boucke,	Pflüg. Arch. ges. Physiol. 256, 43 (1952)
14	H. Breuninger,	Arzneimittel Forsch. 6, 222 (1956)
15	H. C. Brown and C. P. Garg,	J. Am. Chem. Soc. 83, 2952 (1961)
16	A. Cameron and B. N. Craver,	Proc. Soc. exp. Biol. Med. 74, 271 (1950)
17	J. B. Carr,	Univ. of Washington, Ph. D., 1963
18	R. B. Carlin,	J. Am. Chem. Soc. 67, 928 (1945)
19	R. B. Carlin and H. P. Landerl,	J. Am. Chem. Soc. 75, 3969 (1953)
20	F. H. Case, Z. B. Jacobs, R. S. Cook and J. Dickstein,	J. Org. Chem. 22, 390 (1957)
21	A. F. Casey, N. J. Harper and J. R. Dimmock,	J. Chem. Soc. 1964, 3635
22	A. F. Casey,	Tetrahedron 22, 2711 (1966)
23	R. Charlier,	Coronary Vasodilators, Oxford 1961
24	G. Chen,	in: Psychosomatic Medicine, ed. by J. H. Moyer, Philadelphia, 1962
25	W. Cocker, P. V. R. Shannon and P. Stainland,	J. Chem. Soc. C., 1966, 1946
26	R. C. Cookson,	J. Chem. Soc. 1954, 282
27	W. D. Cotterill and M. J. T. Robinson,	Tetrahedron 20, 765 (1964)
28	W. D. Cotterill and M. J. T. Robinson,	Tetrahedron 20, 777 (1964)
29	M. Crawford and F. H. C. Stewart,	J. Chem. Soc. 1952, 4443
30	D. J. Cram and F. A. Abd Elhafez,	J. Am. Chem. Soc. 74, 5828 (1952)
31	D. J. Cram and K. R. Kopecky,	J. Am. Chem. Soc. 81, 2748 (1959)
32	W. G. Dauben, G. J. Foncken and D. S. Noyce,	J. Am. Chem. Soc. 78, 2579 (1956)

33 W. G. Dauben and R. E. Bozak,   J. Org. Chem. **24**, 1596 (1959)
34 D. H. Derbyshire and W. A. Waters,   J. Chem. Soc. **1950**, 573
35 I. R. Dice, L. A. Loveless and   J. Am. Chem. Soc. **71**, 3546 (1949)
    H. L. Cates Jr.,
36 J. R. DiPalma and A. V. Mascatello,   J. Pharmacol. exp. Therap. **101**, 243
       (1951)
37 C. Djerassi and J. Staunton,   J. Am. Chem. Soc. **83**, 736 (1961)
38 G. Drehfahl, G. Heublein and   J. Prakt. Chem. **32**, 69 (1966)
    S. Lochner,
39 E. L. Eliel and R. S. Ro,   J. Am. Chem. Soc. **79**, 5993 (1957)
40 M. N. Rerick and E. L. Eliel,   J. Am. Chem. Soc. **84**, 2356 (1962)
41 E. L. Eliel, N. L. Allinger,   Conformation Analysis, New York 1965
    S. J. Angyal and G. A. Morrison,
42 E. L. Eliel and F. J. Biros,   J. Am. Chem. Soc. **88**, 3334 (1966)
43 Elsevier's Encyclopaedia   Series III, Vol. 12B, page 1356
    of Organic Chemistry   Amsterdam, 1950
44 H. Feltkamp and N. C. Franklin,   Ann. **683**, 55 (1965)
45 H. Feltkamp, N. C. Franklin and   Ann. **683**, 75 (1965)
    W. Kraus,
46 B. O. Field and J. Grundy,   J. Chem. Soc. **1955**, 1110
47 R. F. Furchgott, W. Sleator Jr.   J. Pharmacol. exp. Therap. **129**, 405
    and F. de Gubareff,   (1960)
48 E. W. Garbisch and D. B. Patterson,   J. Am. Chem. Soc. **85**, 3228 (1963)
49 H. L. Goering, R. L. Reeves and   J. Am. Chem. Soc. **78**, 4926 (1956)
    H. H. Espy,
50 H. Goldblatt, J. Lynch, R. F. Hanzal   J. Exp. Med. **59**, 347 (1934)
    and H. W. Summerville,
51 C. Hansch and T. Fujita,   J. Am. Chem. Soc. **86**, 1616 (1964)
52 N. J. Harper, C. F. Chignell and   J. Med. Chem. **7**, 726 (1964)
    G. Kirk,
53 O. Hassel,   Tidsskr. Kjemi. Bergvessen Met. **3**, 32
       (1943)
54 H. Haubenstock and E. L. Eliel,   J. Am. Chem. Soc. **84**, 2363 (1962)
55 P. Heistracher, E. Hell and O. Kraup,   Arzneimittel Forsch. **15**, 146 (1965)
56 G. F. Hennion and F. X. O'Shea,   J. Am. Chem. Soc. **80**, 614 (1958)
57 H. O. House and W. L. Respess,   J. Org. Chem. **30**, 301 (1965)
58 W. Hueckel and H. Naab,   Ber. **64**, 2137 (1931)
59 A. C. Huitric, R. A. Durbin and   J. Am. Pharm. Assoc. Sci. Ed. **48**, 131
    G. H. Bryan,   (1959)
60 A. S. Hussey and J. R. Dyer,   J. Am. Chem. Soc. **73**, 603 (1951)
61 R. C. Huston, W. B. Fox and   J. Org. Chem. **3**, 251 (1938-'39)
    M. N. Binder,
62 L. M. Jackman,   Applications of N.M.R. Spectroscopy,
       Oxford, 1959
63 C. E. Johnson and F. A. Bovey,   J. Chem. Phys. **29**, 1012 (1958)
64 A. V. Kamernitzky and   Zh. Obshch. Khim. **30**, 758 (1960)
    A. A. Akhrem,

65 A. V. Kamernitzky and   Tetrahedron **18**, 785 (1962), translated
    A. A. Akhrem,   from Uspekhi Khimii **1961**, 145
66 N. V. Kaverina,   Pharmacology of the Coronary
                                    Circulation, Oxford, 1966
67 W. A. Klyne,   Experientia **15**, 119 (1956)
68 K. Kochsiek and H. Fritsche,   Arzneimittel Forsch. **16**, 1154 (1966)
69 J. H. Lamneck Jr.,   J. Am. Chem. Soc. **76**, 1106 (1954)
70 O. Langendorff,   Pflüg. Arch. ges. Physiol. **61**, 291 (1895)
71 S. A. Mahood and P. V. L. Shaffner, Org. Synth., Coll. Vol. III, 160 (1943)
72 R. Magnus,   Pflüg. Arch. ges. Physiol. **102**, 123 (1904)
73 J. A. Marshall and R. D. Carroll, J. Org. Chem. **30**, 2748 (1965)
74 F. L. Meyler,   Thesis University of Amsterdam, 1960
75 M. Mouseron, R. Ganger,   Compt. Rend. **224**, 610 (1947)
    F. Winternitz and G. Comber
76 I. N. Nazarov, A. A. Akhrem   Izv. Akad. Nauk S.S.S.R. otdel. Khim.
    and A. V. Kamernitzky,   Nauk **1958**, 631
77 I. N. Nazarov, G. V. Aleksandrova J. Gen. Chem. **28**, 2237 (1958)
    and A. A. Akhrem,
78 Organic Reactions   ed. by R. Adams, volume II, 294,
                                    New York, 1944
79 R. J. Ouellette,   J. Am. Chem. Soc. **86**, 3089 (1964)
80 R. J. Ouellette,   J. Am. Chem. Soc. **86**, 4378 (1964)
81 F. Pristera and M. Halik,   Anal. Chem. **27**, 217 (1955)
82 A. M. Prokhoda, A. V. Kamernitzky Izv. Akad. Nauk. S.S.S.R. otdel. Khim.
    and A. A. Akhrem,   Nauk **1964**, 1060
83 J. Radell, B. W. Brodman and   Can. J. Chem. **43**, 1661 (1965)
    K. Kramer,
84 R. R. Read and D. B. Mullin,   J. Am. Chem. Soc. **50**, 1763 (1928)
85 H. Reichel,   Muskelphysiologie (Lehrbuch der
                                    Physiologie), Berlin, 1960
86 B. Rickborn,   J. Am. Chem. Soc. **84**, 2414 (1962)
87 P. A. Robins and J. Walker,   J. Chem. Soc. **1955**, 1789
88 J. B. Shoesmith and A. Mackie, J. Chem. Soc. **1928**, 2334
89 A. Skita,   Ber. **56**, 1014 (1923)
90 E. E. Smissman and M. Steinman, J. Med. Chem. **9**, 455 (1966)
91 H. H. Smookler and J. J. Defeo, J. Am. Pharm. Assoc. Sci. Ed. **51**, 736
                                    (1962)
92 O. I. Sorokin,   Izv. Akad. Nauk. S.S.S.R. otdel. Khim.
                                    Nauk **1961**, 460
93 D. C. Staif,   University of Washington, Ph. D., 1963
94 M. Tiffeneau, B. Tehoubar,   Bull. Soc. Chim. France **1947**, 445
    Mmme. Saiaslambert and
    Mmme. LeFeillier-Du Pré
95 H. Timmerman, R. F. Rekker and Rec. Trav. Chim. **84**, 1348 (1965)
    W. Th. Nauta,
96 W. Trautwein,   Pharmacol. Rev. **15**, 277 (1963)
97 G. Vavon and J. Conia,   Compt. Rend. **224**, 610 (1947)
98 M. J. G. Waelen,   Thesis University Nijmegen, 1963

98

99   R. Wegria,   Pharmacol. Rev. **3**, 197 (1951)
100 C. Wilson,   in: „Hypertension"; Boerhaave Course,
                                     ed. by J. de Graeff, Leiden 1963
101 M. M. Winbury, P. M. Michiels, J. Pharmacol. exp. Therap. **99**, 343
     W. E. Hambourger, W. J. Stock- (1950)
     fisch and D. L. Cook,
102 N. Winstein and N. J. Holness, J. Am. Chem. Soc. **77**, 5562 (1955)
103 L. N. Yakhontov, D. M. Kras-   J. Gen. Chem. **31**, 2977 (1962)
     nokutskaya and M. Y. Rubtsov
104 W. Ziegenbein, A. Schäffler and Ber. **88**, 1906 (1955)
     R. Kaufhold,
105 B. Siccic and Z. Welvart,   Chem. Comm. **1966**, 499

CURRICULUM VITAE

De auteur van dit proefschrift ontving zijn middelbare school-opleiding aan de Chr. H.B.S. te Emmen (thans Chr. Lyceum); zijn scheikundeleraar was Drs. J. de Wilde.

In 1956 behaalde hij het H.B.S.-B diploma en ving zijn academische studie aan de Vrije Universiteit te Amsterdam aan, in de faculteit der Wiskunde en Natuurwetenschappen. In september 1960 legde hij het kandidaatsexamen af (letter f) en in februari 1964 het doctoraal-examen, met scheikunde als hoofdvak; de hoofdrichting was de organische scheikunde, met uitbreiding in de farmacochemie en als bijvak chemische fysiologie.

In 1960 zette hij als wetenschappelijk ambtenaar aan de V.U. het in dit proefschrift beschreven onderzoek voort, na er gedurende het doctoraal practicum reeds een begin mee gemaakt te hebben, onder leiding van Prof. Dr. W. Th. Nauta.

Het farmacologische gedeelte van het onderzoek is bewerkt in de farmacologische afdeling van het research-laboratorium van de N.V. Koninklijke Pharmaceutische Fabrieken v/h Brocades-Stheeman & Pharmacia, te Haarlem, bij welke onderneming hij in maart 1967 als farmacologisch medewerker op genoemd laboratorium in dienst trad.

100

STELLINGEN

1.

De smeltpunten van enkele uit zuivere ketonen bereide semicarba-
zonen zijn niet constant. Deze derivaten zijn daarom ongeschikt ter
identificatie van carbonylverbindingen. Het goede kristallisatie-
vermogen maakt semicarbazonen wel zeer waardevol om carbonyl-
verbindingen af te scheiden.

> Hoofdstuk II van het chemische deel van dit proefschrift.
> J. Radell, B. W. Brodman en K. Kramer, Can. J. Chem. **43**, 1661
> (1965)

2.

De hypothese van DREFAHL et al. omtrent de conformatie van
de door hen gesynthetiseerde cis-2-piperidinocyclohexanolen wordt
door hun infra-rood spectroscopische gegevens niet bevestigd.

> G. Drefahl, G. Heublein en S. Leuchner, J. Prakt. Chem. **32**, 87
> (1966)

3.

Uit het ontstaan van slechts één isomeer bij de dehydratatie van
3-ethoxymethyl-menthol concludeert DE BOTTON ten onrechte dat het
waarschijnlijke tussenproduct 3-ethoxymethyleen-p-menthaan is.

> M. de Botton, Compt. Rend. **1964**, 4054

4.

De wijze waarop SOROKIN conformaties toekent aan drie door hem
gesynthetiseerde, isomere 1.3.5-trimethyl-4-phenyl-4-piperidinolen
valt sterk te kritiseren.

> O. I. Sorokin, Izv. Akad. Nauk. S.S.S.R. otd. Khim. Nauk. **1961**,
> 460 (Engelse vertaling, pag. 424)

5.

De stelling, dat de aanduidingen „optisch actief" en „optische acti-
viteit" vervangen dienen te worden door de termen „rotatief", res-
pectievelijk „rotativiteit", is aanvechtbaar.

> C. Blomberg, Dissertatie Vrije Universiteit, Amsterdam, 1964. Stel-
> ling IV.

6.

Bij de verklaring van het verschil in Rf waarden bij papier- en
cellulosekolomchromatografie dient men rekening te houden met de
door Weinberg en Keller gevonden effecten.

> B. B. Weinberg en R. A. Keller, J. Chromatog. **16**, 40 (1964)

7.

Vergelijking van biologische eigenschappen binnen een homologe
reeks moet gebaseerd zijn op moleculaire hoeveelheden.

8.

De in de „Toelichting bij -Aanvrage tot inschrijving in het register
van verpakte geneesmiddelen-" onder het hoofd „Farmacologisch-
toxicologisch onderzoek" genoemde punten voor farmacologisch
onderzoek, waarvan bij de aanvrage moet blijken dat ze zijn over-
wogen, zijn aan ernstige bedenkingen onderhevig.

> Toelichting bij Aanvrage tot inschrijving in het register van ver-
> pakte geneesmiddelen. 15 september 1965

9.

Het verdient aanbeveling tijdens de behandeling van asthma-patiënten
met isoprenaline bevattende geneesmiddelen het hart regelmatig aan
de hand van het E.C.G. te controleren.

10.

De wijze waarop volgens de Nederlandse Farmacopee de afwezig-
heid van pyrogenen in injectievloeistoffen moet worden vastgesteld,
levert in sommige gevallen moeilijkheden op.

> Nederlandse Farmacopee, zesde uitgave, tweede druk (1966)

11.

De weerstanden die er bij ouders en kinderen tegen plaatsing op een school voor kinderen met leer- en gedragsmoeilijkheden (L.O.M.) bestaan, zouden voor een groot gedeelte overwonnen kunnen worden door dit onderwijs te doen plaats vinden in symbiose met gewoon lager onderwijs.

12.

De gewoonte om in publieke aanprijzingen van levensmiddelen, geneesmiddelen en genotmiddelen termen als „natuur", „zon" en „land" te bezigen, als zouden deze toverkracht bezitten, is verwerpelijk.