21,20-Anhydromelianone and Melianone from *Simarouba amara* (Simaroubaceae); Carbon-13 NMR Spectral Analysis of Δ^7-Tirucallol-Type Triterpenes

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Abstract. Further examination of the hexane extract of the root bark of *Simarouba amara* (Simaroubaceae) has led to the isolation of two side chain oxygenated Δ^7-tirucallol-type triterpenes in addition to the already reported compounds **4** and **5**. One has been found to be identical with the known melianone **8** and the second is a new compound and has been shown to be 21,20-anhydromelianone **11**. The ^{13}C-NMR spectra of nine Δ^7-tirucallol derivatives have been analysed and the data obtained have been applied to the confirmation of the structure of the new substance **11**.

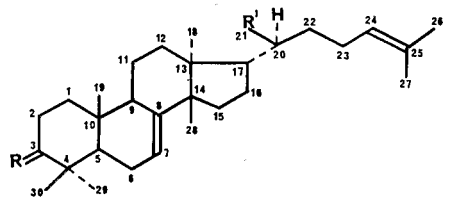
INTRODUCTION

The quassinoids[1,2] and the limonoids (meliacins)[1,3] are groups of structurally complex highly oxygenated triterpene degradation products. They occur in three very closely related plant families. The limonoids which can be considered as modified tertranortriterpenes are found in the Meliaceae and Rutaceae families, and the quassinoids which occur as further triterpene breakdown products (mostly decanortriterpenes) have been found so far only in the Simaroubaceae family.

The triterpenoid biogenetic pathway for the quassinoids has been experimentally verified using labelled mevalonate precursors.[4-6] Biosynthetic work on the limonoid nimbolide has also been reported.[7] As to the triterpenoid precursor of the limonoids and quassinoids, it is currently believed to be a Δ^7-tirucallol-type of triterpene which undergoes an apo-euphol rearrangement.[8,9] This skeletal rearrangement, during which the C_{14} methyl group migrates back to C_8, is supposed to proceed via the 7α,8α-epoxide leading to a 7α-hydroxy-apotirucallol derivative.[10]

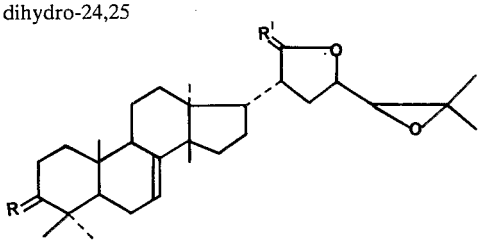
A number of side chain oxygenated Δ^7-tirucallol derivatives, such as flindissol[1], turraeanthin (**7**),[9] melianone (**8**),[11] etc., believed intermediates on the biosynthetic route of the limonoids and quassinoids, have been isolated from the Meliaceae and Rutaceae families. Of particular biogenetic interest are two tirucalla-7-ene derivatives quite recently isolated by Halsall and Troke from *Turraenthus africanus* (meliaceae):[12] the 7α,8α-epoxide of epiturraeanthin and a tirucallane triterpene in which the side chain has been degraded with the loss of four carbon atoms to a γ-lactone and which has been found to be identical with the lactone prepared anteriorly[13] from melianodiol (**12**).

Only a few C_{30}-triterpenes have so far been extracted from the Simaroubaceae family. Melianodiol (**12**) and its diacetate **13** have been isolated[13] from *Samadera madagascariensis* J. (synonymous with *Samadera indica* Gaertn.) and more recently the structure determination of oxo-3-tirucalla-7,24-diene (**4**) and dioxo-3,21-tirucalla-7,24-diene (**5**) from *Simarouba amara* Aubl. has been reported.[14]

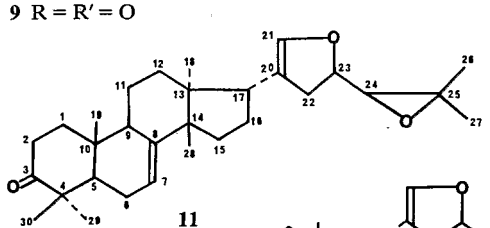


1 R = 3α-OH, H; R′ = CH_3
2 R = 3α-OH, H; R′ = CH_2OH
3 R = 3α-OH, H; R′ = CH_3 — dihydro-24,25

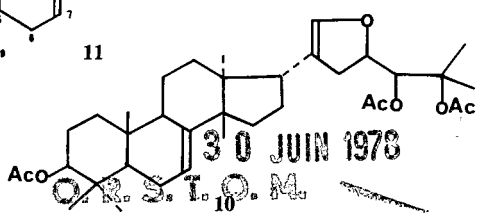
4 R = O; R′ = CH_3
5 R = O; R′ = CHO
6 R = O; R′ = CH_3 — carboxy-26



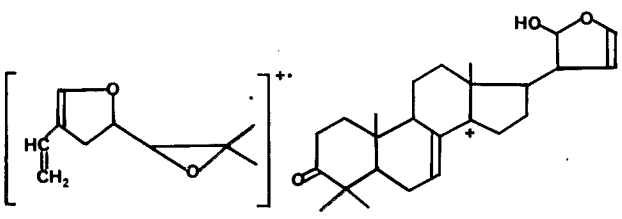
7 R = 3α-OAc, H; R′ = 3α-OH, H
8 R = O; R′ = 3α-OH, H
9 R = R′ = O



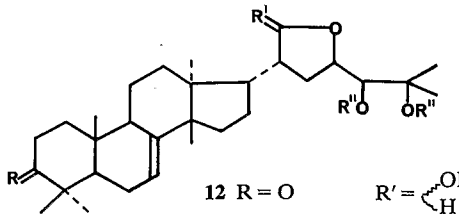
11



10



ion X : $C_{10}H_{14}O_2$ ion Y : $C_{25}H_{33}O_3$



12 R = O R′ = 3α-OH R″ = H

13 R = O R′ = 3α-OH R″ = Ac

14 R = 3α-OAc R′ = 3β-OH R″ = H

Further examination of the root back of *Simarouba amara* from Guyana has now proven the presence of two more Δ^7-tirucallane-type triterpenes. One turned out to be identical with melianone (**8**), the major constituent of *Melia azedarach* (meliaceae), whose structure has been determined by Lavie and co-workers.[11] The second is a new one for which we propose a structure **11** of anhydromelianone.

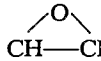
The utility of ^{13}C-NMR spectroscopy for the purpose of structure elucidation of these oxygenated triterpenes of biogenetic interest is evident. Therefore a study and assignment of ^{13}C-NMR shifts in this group of Δ^7-tirucallol derivatives was undertaken and the data obtained have been applied to the confirmation of the structure of the new substance **11**.

20,21-Anhydromelianone **11** *and Melianone* **8**

The powdered root bark of *S. amara* was extracted by percolation with hexane and the extracts chromatographed on silica columns. In this way we isolated, apart from sitosterol, Δ^7-tirucallone (**4**) and 21-oxo-Δ^7-tirucallone (**5**) previously obtained from the stem bark, two crystalline compounds.

20,21-Anhydromelianone (**11**). Crystals from methanol, mp 165–166°, $[\alpha]_D$-34.2° (c = 0.94; $CHCl_3$). It has the formula $C_{30}H_{44}O_3$ (found: M^+ 452.3299; calcd 452.3290). The triterpenoid nature of this compound is indicated by its ^{1}H-NMR spectrum which displays seven quaternary methyl signals [at 0.78, 1.02, 1.07 (6H), 1.12, 1.32 and 1.35 ppm]. The IR spectrum (nujol) which has no OH bands, shows a band at 1700 cm^{-1} for a six-membered ring ketone and the CD curve in dioxane shows a negative Cotton effect ($\Delta\varepsilon$ = 0.63 at 297 nm) of the same sign and magnitude as that of 3-keto-Δ^7-triterpenoids.[11,15]

The presence of the 24,25-epoxy group is indicated by the following: (a) Two of the methyl groups appear in the ^{1}H-NMR spectrum at chemical shifts (δ 1.32 and 1.35 ppm) which suggest that they are attached to a carbon atom bearing oxygen; (b) This NMR spectrum has a signal (doublet) at 2.91 ppm (J = 8 Hz) due to a proton of an epoxide ring; (c) A peak at m/e 381 having the fragment formula $C_{26}H_{37}O_2$ accounts for the loss of the



CH—CMe_2 group. These spectral data are in good agreement with those observed for a similar epoxy group present in aglaiol,[16] turraeanthin (**7**)[9] and melianone (**8**).[11]

The ^{1}H-NMR spectrum (and the ^{13}C-NMR spectrum, see later) shows the presence of two double bonds, each carrying one vinyl proton. The chemical shift and the multiplicity of one of them (m at δ 5.36 ppm) agrees with the presence of a $\Delta^{7,8}$ double bond.[14]

The dihydrofuran structure on the side chain as shown in **11**, which accounts for the second double bond and for the third oxygen atom, was disclosed from the following arguments. The ^{1}H-NMR spectrum of **11** shows a one proton signal at 6.12 ppm (d, ^{4}J = 1.5 Hz) and a one proton multiplet centered at 4.35 ppm. These chemical shifts fit well for the C_{21} proton and the C_{23} proton, respectively; the signal at 4.35 ppm was an ill-defined six-line pattern, the C_{23} proton being coupled to the C_{24} proton and further split by the C_{22} methylene protons. Furthermore, the mass spectrum of **11** showed the base peak at m/e 166 having the fragment formula $C_{10}H_{14}O_2$ (found: 166.0984; calc 166.0985), which can reasonably be assigned to the ion (X) arising by cleavage of the C_{13}–C_{17} and C_{15}–C_{16} bonds.

An attempt to correlate compound **11** to turraeanthin **7** by treatment of the latter with mesyl chloride in pyridine failed. But refluxing turraeanthinglycol (**14**)[17] with acetic anhydride in presence of sodium acetate led, in 25% yield, to the non-previously described compound **10**: Crystals from methanol, mp 133–136°, $[\alpha]_D$-17° (c = 1.19 $CHCl_3$), $C_{36}H_{54}O_7$ (M^+ 598).* It's IR spectrum had no OH band and its ^{1}H-NMR spectrum shows signals for the C_{26} and C_{27} methyl groups at 1.63 and 1.53 ppm, for three acetoxy-groups (δ 1.96, 2.07 and 2.15 ppm) and for two single protons at δ 5.26 ppm (H_7) and at 6.0 ppm (d, ^{4}J = 0.6 Hz). The latter signal is assigned to the C_{21} proton as shown by it ^{13}C-NMR spectrum (Table 1) which displays four olefinic sp^2 carbon atoms: C_7 (d, 118.0 ppm), C_8 (s, 145.5 ppm), C_{20} (s, 115.4 ppm) and C_{21} (d, 140.2 ppm).

Lack of material has prevented the conversion of 21,20-anhydromelianone to compound **10**, but its structure is fully confirmed by its ^{13}C-NMR spectrum and particularly by comparison with that of compound **10**.

Melianone (**8**). The more polar compound crystallised from chloroform-pentane, mp 212–214°, $[\alpha]_D$-48° (c = 0.97; $CHCl_3$). It has the formula $C_{30}H_{46}O_4$ (found: M^+ 470.3415; calcd 470.3396). Its IR spectrum, which showed an OH band and a carbonyl band (1700 cm^{-1}), and its ^{1}H-NMR spectrum indicated this compound to be melianone (**8**).[11] Oxidation with chromium trioxide-pyridine gave the γ-lactone **9**, $C_{30}H_{44}O_4$ (M^+· 468), mp 197–199°, $[\alpha]_D$-71.5° (c = 0.92; $CHCl_3$), with infrared bands at 1700 and 1775 cm^{-1}, thus proving the presence of the five-membered cyclic hemiacetal structure. The ^{1}H-NMR spectrum (60 MHz) of compound **8** displays a set of signals between 0.85 and 1.34 ppm which can be related to seven methyl groups: A doublet (1 H) at 2.9 ppm (J = 8 Hz) for the epoxide proton (24-H), a multiplet (1 H) centered at 3.95 ppm for the C_{23} proton, and a non resolved triplet (2 H) at 5.38 ppm for the C_7 and C_{21} protons. The ^{1}H-NMR spectrum (90 MHz) of the lactone **9** shows seven distinct methyl signals (δ 0.83, 1.02, 1.04, 1.07, 1.12, 1.35 and 1.37 ppm) and three one proton signals, a doublet at 2.81 (J = 7.6 Hz) and multiplets at

* Chemical ionization mass spectrum shows a quasi molecular peak MH^+ at m/e 599.

Table 1. Carbon Chemical Shifts

Carbon number	1	2	3	4	5	6	7	8	9	10	11
1	37.1	37.2	37.1	38.4	38.2	38.3	36.7	38.5	38.5	36.8	38.5
2	27.6	27.6	27.6	34.9	34.7	34.7	23.8	35.0	35.1	23.8	35.1
3	79.0	78.9	78.8	216.6	215.4	216.6	81.0	216.8	216.7	81.0	216.5
4	38.8	38.8	38.8	47.8	47.6	47.6	37.7	47.8	47.8	37.8	47.8
5	50.5	50.5	50.5	52.3	52.1	52.1	50.7	52.4	52.4	50.8	52.3
6	23.9	23.8	24.1	24.4	24.7	24.3[a]	23.2	24.4	24.4	23.8	24.4
7	117.3	117.6	117.3	117.7	117.8	117.6	117.8	118.0	118.2	118.0	118.1
8	145.3	145.0	145.3	146.0	144.6	145.7	145.7 145.5	145.6	145.3	145.5	145.4
9	48.9	48.7	48.8	48.2	48.2	48.3	48.6 49.5	48.3 49.5	48.4	48.8	48.4
10	35.8	34.8	34.8	34.9	34.9	34.7	34.8	34.9	34.9	34.9	34.9
11	27.2	27.3	27.6	27.3	26.9	27.3	27.4	27.4	27.4	27.4	27.4
12	34.0	33.9	33.9	34.0	31.9	33.4	35.1	35.1[a]	33.8[a]	36.8	36.2
13	43.4	43.2	43.3	43.5	43.2	43.4	43.5	43.5	43.7	44.1	44.1
14	51.0	51.0	50.9	51.1	50.7	50.9	50.4	50.4	50.5	50.3	50.2
15	33.8	32.8	33.9	33.6	33.6	32.8	34.2	34.2[a]	31.0[a]	34.3	34.3
16	28.1	24.8	27.6	28.2	29.1	27.9	31.5	31.5[a]	30.0[a]	31.9	31.9
17	52.8	46.9	52.8	52.9	48.2	52.5	46.9 45.1	46.9 45.1	47.1	45.4	45.3
18	13.1	13.1	13.1	12.7	12.7	12.6	13.0	12.7	12.7	13.1	12.7
19	18.1	17.7	18.0	18.3	17.7	18.3	17.5	17.7	17.6	17.5	17.7
20	36.1	42.5	36.0	35.8	55.0	35.8	33.7	33.8	40.4	115.4	114.0
21	18.1	62.3	18.0	18.3	204.7	18.3	101.6 97.6	101.7 97.7	177.6	140.2	140.0
22	36.1	29.5	36.0	36.1	29.6	35.4	24.9	24.9	23.5	25.2	24.9
23	25.0	25.6	24.9	24.9	25.8	26.9[a]	78.3 77.1	78.4 77.0	77.9	76.6	80.9
24	124.7	124.5	39.3	125.1	123.1	125.9	67.8 65.4	67.4 65.4	64.5	78.5	65.7
25	130.3	130.8	27.6	130.8	131.9	146.6	57.8 57.1	57.9 57.2	57.2	82.9	57.2
26	25.6	25.6	22.5	25.6	25.5	173.0	24.1[a]	24.9[a]	23.5[a]	24.2[a]	25.3[a]
27	17.6	17.7	22.5	17.5	17.7	20.3	19.2[a]	19.2[a]	19.4[a]	22.9[a]	19.3[a]
28	21.8	22.0	21.5	21.5	22.8	21.3	22.5	22.5	23.0	22.9	22.0
29	27.6	27.6	27.6	24.4	24.3	24.3	27.4	24.4	24.4	27.4	24.4
30	14.3	14.7	14.6	21.5	21.5	21.4	15.8	21.5	21.5	15.8	21.5

a. Values within any vertical column may be reversed. In compound **7**: $CH_3\underline{CO}$ at 171.05 ppm; $\underline{CH_3}CO$ at 21.5 ppm. In compound **10**: CH_3CO at 170.9; 170.6 and 170.1 ppm; CH_3CO: 22.4; 21.3; 21.0 ppm.

4.16 and 5.30 ppm, due to the C_{24}, C_{23} and C_7 protons, respectively. The assignment of the signals to C_{24} and C_{23} protons was demonstrated by a spin decoupling experiment.

Final proof of the structure **8** was obtained by direct comparison with authentic melianone from *Melia azedarach*[11] (identity of MS, ^1H and ^{13}C-NMR spectra).

The ^{13}C-NMR spectrum of melianone (see below) shows clearly that it exists in solution like turreaenthin (**7**),[9] as a C_{21} epimeric mixture. This may explain the discrepancy of the mp and $[\alpha]_D$ we observed and those quoted in Refs. 7 and 9.

As to the fragmentation pattern of melianone by mass spectroscopy, its high resolution mass spectrum does not show any peak at m/e 71 reported to arise by cleavage of the C_{24}–C_{23} bond but rather shows a weak peak at m/e 399 (11.2%) corresponding to the composition $C_{25}H_{33}O_2$, (M^+–71). The base peak is observed at m/e 383.2586 having the fragment formula $C_{25}H_{35}O_3$ which can be assigned to the ion (Y). Other significant peaks are found at m/e 437 (83%) $C_{29}H_{41}O_3$ (M^+–Me–H_2O), m/e 365 (70.4%) $C_{25}H_{33}O_2$ (ion Y–H_2O) and at m/e 297 (46%) $C_{21}H_{29}O$ (ion Y–$C_4H_6O_2$).

*Carbon-13 NMR Analysis of Compounds **1** to **11***

Noise and single frequency decoupled ^{13}C-NMR spectra were recorded* for compounds **1** to **11** and the chemical shift assignments are indicated in Table 1. The carbon resonances in these compounds were assigned on the basis of the residual splitting in off-resonance decoupled spectra, data of the previously published models,[18–21] and comparison with the spectra of structurally related compounds. The structure determination of compounds **4** and **5** and the preparation of their derivatives **1**, **2** and **3** have been reported previously;[14] the ^{13}C-NMR spectrum of terebinthone (**6**) was obtained by courtesy of Professor E. Wenkert and Dr. J. de Paiva de Campello.

The chemical shift assignment for compounds **1** to **9** is straightforward and does not require any special comment. The C_2 and C_{10} resonances in compound **4** are found at 34.9 ppm as a signal which appears as a triplet (overlapped by a singlet) in the off-resonance decoupled

* ^{13}C-NMR spectra were recorded in $CDCl_3$ solution on a Bruker HX-90E F.T. NMR spectrometer operating at 22.63 MHz. Chemical shifts are given in ppm with respect to internal TMS.

spectrum (sford), whereas, it is found as a singlet due to the C_{10} carbon in the sford spectrum of the 2-dideuterated derivative. Inspection of the chemical shifts for the side chain carbons of the presently reported compounds **1–6** and for the previously described euphol[19] indicates that the C_{20} stereochemistry cannot be deduced from ^{13}C-NMR spectroscopy. The chemical shifts for the two series of substances are identical within the experimental error as far as the C_{17} side chain carbons are concerned.

The proposed C_{17} side chain of our new compound **11** is in good agreement with the observed chemical shifts. The δ-values of 65.7 (d) and 57.2 (s) ppm reflect respectively the presence of a neopentylic epoxymethine and a quaternary epoxy carbon; they are also found in turreaenthin (**7**) and melianone (**8**) which possess the C_{24}–C_{25} epoxide ring. This is further confirmed by the shift contrast between **11** and **10**, since in the spectrum of the latter compound C_{24} and C_{25} appear at 78.5 and 83.9 ppm. The C_{23} resonance in compound **11** appears at 80.9 ppm (d), which is shifted to about 2 ppm highfield in turreaenthin and melianone.

The spectrum of compound **11** clearly shows one carbonyl (s at 216.5 ppm) and four olefinic sp^2 carbon atoms: C_7 (d, 118.1 ppm), C_8 (s, 145.4 ppm), C_{20} (s, 114.0 ppm) and C_{21} (d, 140.0 ppm).

Inspection of the ^{13}C spectra of turreaenthin (**7**) and melianone (**8**) reveals that these compounds are epimeric mixtures at C_{21}. Actually, these spectra reveal two signals for the C_{21} carbon atom while there is only one present for C_{21} in the spectra of the compounds **9**, **10** and **11**, which do not possess the hemiacetal structure. As a result of the epimeric mixture examined, important differences can be noticed for the carbon signals which are in the vicinity of C_{21}. Concerning turreaenthin, Halsall and coworkers[9] had reported that turreaenthin gives rise to an equilibrium mixture of the two possible hemiacetal structures, (the hemiacetal hydroxyl group being *cis* or *trans* to the four-carbon unit carrying the epoxy-group) and that this affects the chemical shift of the C_{24} proton. It is of interest to note that some carbons far from the C_{21} site also show closely spaced double signals as a consequence of subtle conformational effects.

The structure of 21–20 anhydromelianone (**11**) is thus confirmed by the various ^{13}C-NMR spectra examined during this investigation and particularly by the near identity of the chemical shifts of all the carbon atoms except those involved in, or in the immediate environment of, the epoxide and ketone of **11** and the acetoxy groups of **10**.

^{13}C-NMR analysis of the quassinoids[22] and limonoids[23] have been recently published.

Acknowledgement. We are greatly indebted to Professor M. G. Halsall for samples of turreaenthin and turreaenthin-diol and to Professor D. Lavie for a sample of melianone. We wish to thank Mr. C. Marazano for high resolution mass measurements, Mr. P. Varenne for chemical ionisation mass spectrum, Mrs. C. Fontaine and Mr. B. Septe for recording the NMR spectra and Dr. G. Lukacs for his help in their interpretation.

REFERENCES

1. J. D. Connolly, K. H. Overton and J. Polonsky in L. Reinhold and Y. Liwschitz, eds., *Progress in Phytochemistry*, Vol. II, p. 385, Interscience Publishers, London, 1970.
2. J. Polonsky in W. Herz, H. Grisebach and G. W. Kirby, eds., *Progress in the Chemistry of Organic Natural Products*, vol. 30, Springer, Wien, New York, 1973, p. 101.
3. D. L. Dreyer in L. Zechmeister, ed., *Progress in the Chemistry of Organic Natural Products*, vol. 26, Springer, Wien, New York, 1968, p. 190.
4. J. Moron, J. Rondest and J. Polonsky, *Experientia*, **22**, 511 (1966).
5. J. Moron and J. Polonsky, *Tetrahedron Lett.*, 385 (1968).
6. J. Moron, A. Merrien and J. Polonsky, *Phytochemistry*, **10**, 585 (1971).
7. D. E. U. Ekong, S. A. Ibiyemi and E. O. Olagbemi, *Chem. Commun.*, 1117 (1971).
8. D. Arigoni, D. H. R. Barton, E. J. Corey, O. Jeger, L. Caglioti, S. Dev, P. G. Ferrini, E. R. Glazier, A. Melera, S. K. Pradhan, K. Schaffner, S. Sternhell, J. F. Templeton and S. Tobinaga, *Experientia*, **16**, 41 (1960).
9. C. W. L. Bevan, D.E. U. Ekong, T. G. Halsall and P. Toft, *J. Chem. Soc.* (*C*), 820 (1967).
10. G. P. Cotterell, T. G. Halsall and M. J. Wriglesworth, *Chem. Commun.*, 1121 (1967).
11. D. Lavie, M. K. Jain and I. Kirson, *J. Chem. Soc.* (*C*), 1347 (1967).
12. T. G. Halsall and T. A. Troke, in *Abstract Book of 10th IUPAC Symposium of Natural Products*, New Zealand, 1976.
13. A. Merrien and J. Polonsky, *Chem. Commun.*, 261 (1971).
14. J. Polonsky, Z. Baskevitch-Varon and B. C. Das, *Phytochemistry*, **15**, 337 (1976).
15. D. Lavie, K. M. Jain and I. Kirson, *Tetrahedron Lett.*, 2049 (1966).
16. D. Shiengthong, A. Verasam, P. Nanonggai-Suwanrata and E. W. Warnhoff, *Tetrahedron*, **21**, 917 (1965).
17. J. G. St. C. Buchanan and T. G. Halsall, *Chem. Commun.*, 48 (1969).
18. G. Lukacs, F. Khuong-Huu, C. R. Bennett, B. L. Buckwalter and E. Wenkert, *Tetrahedron Lett.*, 3515 (1972).
19. S. A. Knight, *Tetrahedron Lett.*, 83 (1973).
20. H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, **91**, 7445 (1969).
21. H. Eggert and C. Djerassi, *J. Org. Chem.*, **38**, 3788 (1973).
22. J. Polonsky, Z. Baskevitch, H. E. Gottlieb, E. W. Hagaman and E. Wenkert, *J. Org. Chem.*, **40**, 2499 (1975).
23. D. A. H. Taylor, *J. Chem. Res.*, **2**, 14 (1977).