CONSTITUTION OF MAXIMA SUBSTANCE B

By S. Rangaswami, F.A.Sc. and B. V. Rama Sastry*

(Department of Pharmacy, Andhra University, Waltair)

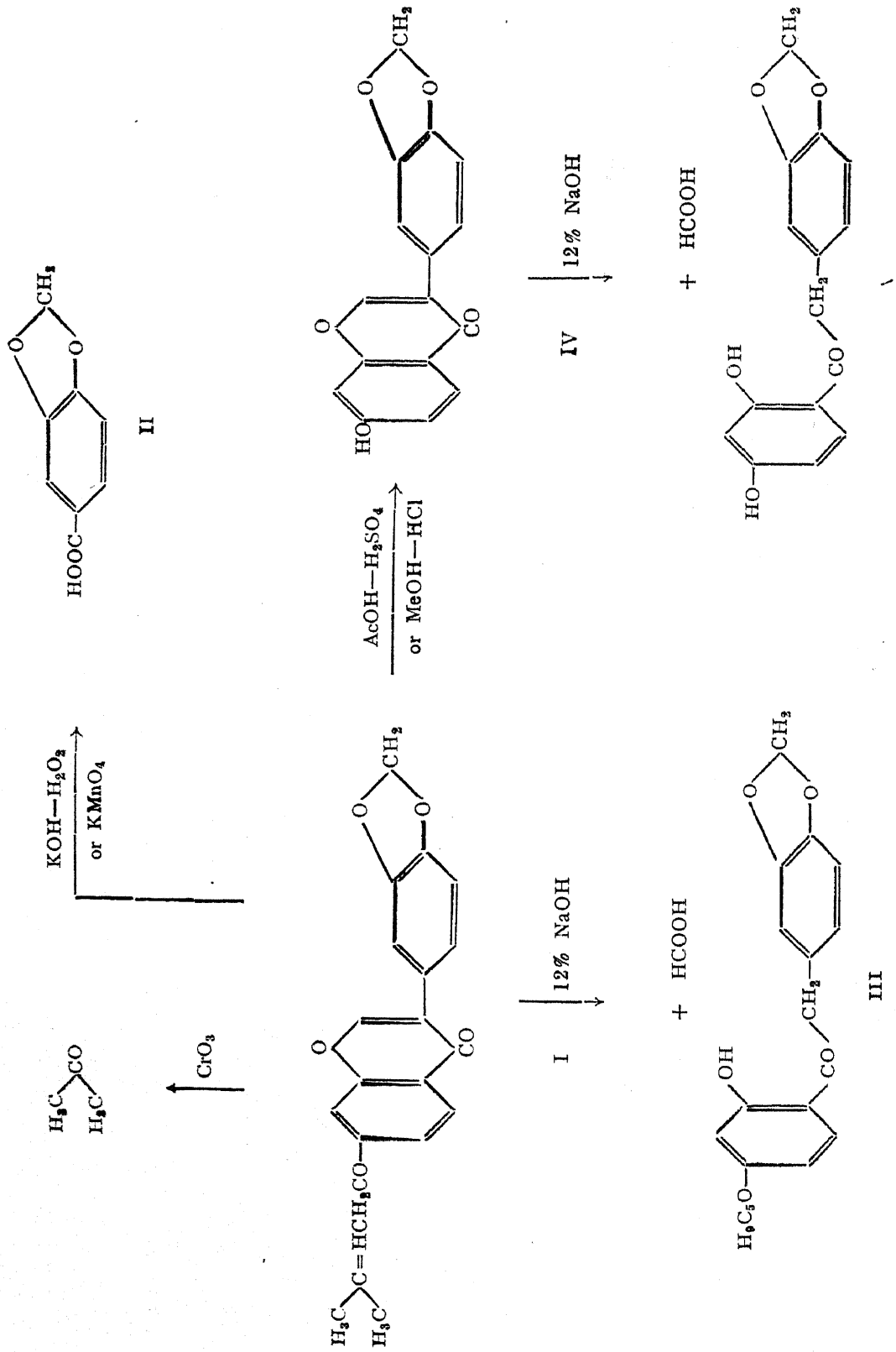
Received January 11, 1963

The constitution of maxima substance B, a substance isolated from the roots of *Tephrosia maxima* Pers. as 7-0-γ: γ-dimethylallyl-pseudobaptigenin (I) was indicated in a brief preliminary note from these laboratories a few years back.[1] A substance having this structure has recently been synthesized by Kukla and Seshadri and its identity with natural maxima substance B has been reported by them in a brief preliminary note.[2] Since no details of the natural product and its derivatives and degradations have so far been reported, the same are described herein.

Maxima substance B is a neutral substance, insoluble in alkali, giving no ferric colour and unaffected under acetylating conditions (sodium acetate and acetic anhydride at 145° for 3 hours). It is optically inactive. That it is an isoflavone was clear from the liberation of formic acid and an *o*-hydroxy-ketone (III) on hydrolysis with sodium hydroxide, and an aromatic acid (piperonylic acid) on treatment with alkaline hydrogen peroxide or potassium permanganate. Treatment with mineral acids under mild conditions resulted in the splitting off of a C_5-unit and formation of a phenolic substance (IV) which could be acetylated and alkylated under the usual conditions. That no change other than the dealkylation of an ether had taken place under the influence of mineral acids was clear from the properties and reactions of the phenolic substance, which also gave formic acid and an *o*-hydroxyketone on alkaline fission. The properties of the phenol (IV), its acetate, methyl and ethyl ethers and the hydroxyketone (V) led to the recognition of (IV) as pseudobaptigenin and of (V) as pseudobaptigenetin. This deduction was confirmed by direct comparison with synthetic substances (pseudobaptigenin and pseudobaptigenetin) prepared according to the method of Baker *et al.*[3]

The identity of the phenol (IV) having been established as pseudobaptigenin, the identity of maxima substance B (non-phenolic) had to be inferred

* Present address: Department of Pharmacology, Vanderbilt University, Nashville-5 (Tenn.), U.S.A.


II
IV
12% NaOH
+ HCOOH
AcOH—H2SO4
or MeOH—HCl
KOH—H2O2
or KMnO4
CrO3
I
12% NaOH
+ HCOOH
III

as an ether of (IV) with the alkyl attached to the only available phenolic group (at 7), and the nature of the alkyl residue (C_5H_9) should be such that dealkylation takes place under the very mild conditions employed. Such facile dealkylation had been noticed earlier with imperatorin (a furocoumarin derivative),[4] in which the alkyl residue so split off is γ:γ-dimethylallyl. The alkyl unit split off from maxima substance B (*viz.*, C_5H_9) exactly fits in with its probable identity as a γ:γ-dimethylallyl. Confirmatory evidence for this point was obtained by oxidising maxima substance B with chromic acid, whereby a volatile ketone was liberated whose identity as acetone was established by preparing the dinitrophenylhydrazone and direct comparison with authentic material. Hence maxima substance B can be represented as (I).

The natural occurrence of a dimethylallyloxyisoflavone (this being the first reported case) has considerable interest from the point of view of the biogenesis of the rotenone group of compounds. This point has been already discussed by the authors in connection with the constitution of a slightly more complicated but closely related substance, *viz.*, maxima substance C.[5]

Experimental

Maxima substance B (I).—For isolation *see* reference 6. The substance crystallised as colourless plates from alcohol, m.p. 126–28°. It dissolved in concentrated sulphuric acid giving an orange-coloured solution which turned yellowish brown in 15 min. On dilution with acetic acid or water no fluorescence was observed. A green colour was produced on warming with gallic acid and sulphuric acid (test for methylenedioxy group). It did not answer rotenoid colour reactions. The substance was unaffected when boiled in alcoholic solution with iodine and sodium acetate (Found: C, 72·2; H, 5·5; —OCH_3, nil. $C_{21}H_{18}O_5$ requires: C, 72·0; H, 5·2%).

Hydrolysis with alkaline hydrogen peroxide.—Isolation of piperonylic acid (II).—A solution of maxima substance B (250 mg.) in alcohol (20 ml.) was treated with aqueous potassium hydroxide (5 ml. of 25% solution), the solution warmed and further treated with 30% hydrogen peroxide added little by little with continuous shaking (total 3 ml.). After the effervescence ceased, the solution was warmed on a water-bath to decompose excess of hydrogen peroxide. The resulting solution was diluted with water (100 ml.), acidified with dilute hydrochloric acid and extracted with ether. The ether extract was shaken with sodium bicarbonate and sodium hydroxide solutions to separate acidic and phenolic components. The acidic fraction recovered in the usual manner (144 mg.) gave on repeated crystallisation from benzene-acetone and benzene colourless plates, m.p. 226–28° (decomp.). It did not

give any colour with ferric chloride, but gave a positive test for methylenedioxy grouping (Found: C, 58·2; H, 4·0. $C_8H_6O_4$ requires: C, 57·9; H, 3·6%). Mixed m.p. with authentic piperonylic acid was undepressed.

The phenolic and neutral hydrolytic products recovered as usual did not crystallise well and were not examined further.

Hydrolysis with 12% sodium hydroxide.—Formation of formic acid and isolation of hydroxyketone (III).—To an alcoholic solution of maxima substance B (590 mg. in 20 ml.) a hot solution of sodium hydroxide (6 gm. in 7 ml. alcohol and 23 ml. water) was added and the mixture was refluxed for 15 min. The solution was cooled, diluted with water (100 ml.), rendered acidic to congo red with 20% phosphoric acid, extracted with petroleum ether (5 × 100 ml.) and the petroleum ether solution was washed with water.

The aqueous acid solution and aqueous washings of the petroleum ether solution were combined and distilled until the contents were reduced to about 50 ml. The distillate was titrated with standard sodium hydroxide solution using phenolphthalein as indicator to estimate the formic acid contained in it; a blank experiment was also carried out. Formic acid found: 56% of theoretical. The neutralised distillate was reduced in bulk to 100 ml. by evaporation and the formic acid contained in an aliquot portion determined by Reisser's method[7] (treatment with mercuric chloride reagent and estimation of the mercurous chloride formed by adding known excess of standard iodine solution and back titration of excess iodine with standard thiosulphate solution). Total formic acid estimated by this method was 54·8% of theoretical. Another portion of the neutralised distillate was tested for formic acid qualitatively by reduction with magnesium and sulphuric acid and testing for formaldehyde by standard qualitative reactions.

The petroleum ether solution mentioned above was dried over sodium sulphate and evaporated. The residue was taken up in ether and the ether solution washed with aqueous sodium bicarbonate to remove acidic components. The residue obtained on evaporating the ether gave on repeated crystallisation from methyl alcohol colourless needles, m.p. 73–74°. This substance gave an yellowish brown colour with alcoholic ferric chloride, a pink solution with conc. sulphuric acid and a positive test for the methylenedioxy group [Found: C, 71·2; H, 6·3; $C_{20}H_{20}O_5$ (ketone III) requires: C, 70·6; H, 5·9%]. The 2:4-dinitrophenylhydrazone of this ketone prepared as usual crystallised from chloroform-methanol as deep red plates,

m.p. 197–99° (Found: C, 60·8; H, 5·0; N, 11·2. $C_{26}H_{24}O_8N_4$ requires: C, 60·0; H, 4·6; N, 10·8%).

The sodium bicarbonate washings mentioned above on acidification and extraction with ether did not yield any crystalline products.

Action of mineral acids on maxima substance B — Formation of Pseudobaptigenin (IV): (*a*) *Using acetic acid and sulphuric acid.* The substance (200 mg.) dissolved in glacial acetic acid (4 ml.) was treated with conc. sulphuric acid (1 ml.) and the mixture maintained at 60–70° for 15 min. The colour of the solution changed from yellow to red and finally to dark reddish brown. The solution was cooled and poured into excess of ice-water and the precipitate obtained was filtered, washed, dried and crystallised from methyl alcohol and then from acetone. Colourless microcrystalline powder, m.p. 293–95°, was obtained.

(*b*) *Using methyl alcoholic hydrogen chloride.* The substance (200 mg.) was refluxed with 10% methyl alcoholic hydrogen chloride (25 ml.) for 2 hours and poured into excess of ice-water. The product obtained crystallised from methyl alcohol and acetone as colourless microcrystalline powder, m.p. 290–93°, identical with the product of experiment (*a*).

The product, m.p. 293–95°, mentioned above was sparingly soluble in the common organic solvents, insoluble in sodium bicarbonate solution, but soluble in sodium hydroxide solution forming a yellow solution. There was no colour with alcoholic ferric chloride or with Wilson's boric acid-citric acid reagent.[8] The test for methylenedioxy group was positive [Found: C, 68·7; H, 4·0. $C_{16}H_{10}O_5$ (IV) requires: C, 68·1; H, 3·6%]. Mixed m.p. with synthetic pseudobaptigenin was undepressed.

The acetate of (IV) prepared with sodium acetate and acetic anhydride crystallised from methanol-acetone as colourless needles, m.p. 164–65° (Found: C, 67·0; H, 4·0. $C_{18}H_{12}O_6$ requires: C, 66·7; H, 3·7%).

The methyl ether of (IV) (prepared using dimethyl sulphate, acetone and potassium carbonate) crystallised from methanol-acetone as colourless prisms, m.p. 180–81° [Found: C, 68·8; H, 4·4; $-OCH_3$, 10·2. $C_{17}H_{12}O_5$ requires: C, 68·9; H, 4·1; $-OCH_3$ (1), 10·5%].

The ethyl ether of (IV) (prepared using diethyl sulphate, acetone and potassium carbonate) crystallised from acetone-ether as colourless prisms, m.p. 168–69° [Found: C, 69·7; H, 4·8; $-OC_2H_5$, 14·8. $C_{18}H_{14}O_5$ requires: C, 69·7; H, 4·5; $-OC_2H_5$ (1), 14·5%].

Hydrolysis of (IV) with 12% sodium hydroxide.—Formation of formic acid and isolation of pseudobaptigenetin (V).—The hydrolysis, etc., were carried out exactly as described in the corresponding experiment with maxima substance B. Formic acid found was 52% in both methods of estimation, *viz.*, titration with standard alkali and estimation by Reisser's method. The ketonic fission product isolated as in the experiment with maxima substance B crystallised from acetone-benzene as colourless prisms, m.p. 146–48°. It gave a wine-red colour with alcoholic ferric chloride, and a positive test for methylenedioxy grouping [Found: C, 66·7; H, 4·8. $C_{15}H_{12}O_5$ (V) requires: C, 66·2; H, 4·4%]. Mixed m.p. with synthetic pseudobaptigenetin was undepressed. The 2, 4-dinitrophenyl hydrazone prepared as usual crystallised from methanol-ethyl acetate as bright red plates, m.p. 254–55°, undepressed by admixture with authentic material (Found: C, 55·4; H, 4·0; N, 12·0. $C_{21}H_{16}O_8N_4$ requires: C, 55·8; H, 3·6; N, 12·4%).

Action of chromic acid on maxima substance B.—Detection of acetone.—The substance (300 mg.) was dissolved in glacial acetic acid (10 ml.), treated with chromic acid solution (0·4 gm. of chromic anhydride in 4 ml. glacial acetic acid and 4 ml. water) and the mixture allowed to stand well-stoppered at room temperature for 40 hours. The dark-brown solution was rendered alkaline with 10% sodium hydroxide added dropwise under efficient cooling with ice. From the alkaline solution 20 ml. were carefully distilled off, and the distillate redistilled to collect 5 ml. of second distillate. This was treated with 2, 4-dinitrophenylhydrazine (50 mg. in 2·5 ml. alcohol) and 2 drops of hydrochloric acid. The DNP derivative that slowly separated was repeatedly crystallised from dilute alcohol yielding yellow needles, m.p. 108–10°, undepressed by admixture with authentic DNP derivative of acetone, but showing considerable depression on admixture with authentic DNP derivative of methyl ethyl ketone (Found: C, 44·9; H, 4·4. $C_9H_{10}O_4N_4$ requires: C, 45·4; H, 4·2%).

SUMMARY

Reactions and degradations leading to the constitution of maxima substance B as 7-0-γ: γ-dimethylallyl-pseudobaptigenin (I) are described.

REFERENCES

1. Rangaswami, S. and Sastry, B. V. R. — *Curr. Sci.*, 1955, **24**, 337.
2. Kukla, A. S. and Seshadri, T. R. — *J. Sci. Ind. Res., India*, 1962, **21 B**, 97.

3. Baker, W., *et al.* .. *J. Chem. Soc.*, 1953, 1852.
4. Späth, E. and Klager, K. .. *Ber.*, 1933, **66**, 914.
5. Rangaswami, S. and Sastry, B. V. R. *Arch. Pharm.*, 1959, **292**, 170.
6. Rangaswami, S., *et al.* .. *Ind. J. Pharm.*, 1961, **23**, 31.
7. Reisser .. *Z. Physiol. Chem.*, 1916, **96**, 355.
8. Wilson, C. W. .. *J. Am. Chem. Soc.*, 1939, **61**, 2303.