CONSTITUTION OF VOGELETIN

The Pigment from the Seeds of Tephrosia vogelii Hook.

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The seeds of Tephrosia vogelii Hook. (Fam.: Leguminosae) grown in the Nilgiris, South India, were examined by Rangaswami and Sastry. Besides the rotenoid compounds tephrosin, deguelin and dehydrodeguelin, they reported the isolation of a new substance which answered some of the colour reactions characteristic of the anthoxanthin group of compounds. Chemical work on the constitution of this substance, which is now given the name vogeletin, is described in this paper.

On the basis of the several degradation and other reactions described in the sequel, the molecular formula of vogeletin is now revised to C_{16}H_{12}O_{7}. It is soluble in alkali and gives a deep green colour with alcoholic ferric chloride and an orange precipitate with lead acetate. It contains a methoxyl group and is free from methylenedioxy groups. It gives a pink colour on reduction with magnesium and hydrochloric acid, zinc and hydrochloric acid or sodium amalgam. It gives a positive reaction in Wilson's boric acid test. These reactions indicate that vogeletin may be a hydroxy flavone or flavonol with free hydroxyl groups and a methoxyl group and that position 5 is probably occupied by a hydroxyl or a methoxyl.

Vogeletin forms a tetracetate with acetic anhydride and sodium acetate and a tetramethyl ether with dimethyl sulphate and potassium carbonate indicating that it is a tetrahydroxy compound. Since the same methyl ether was produced even when diazomethane was employed as the methylating agent, it can be concluded that there is no specially resistant hydroxyl group. This point is referred to again in the sequel.

Information regarding the positions of the substituent groups was obtained from alkaline fission experiments. Vogeletin itself proved resistant to the mild alkaline conditions employed by us, but O-tetramethylvogeletin underwent smooth cleavage. One of the fission products was anisic acid (IV), which proved that the side phenyl nucleus of the anthoxanthin carries only one methoxyl group and that it is in the 4'-position. The other fission product answered the colour reactions of an ortho-hydroxy
aromatic ketone. Its analysis corresponds to $C_{12}H_{16}O_6$ with 4 methoxyl groups (III). It was characterised as its 2:4-dinitrophenylhydrazone and it gave a monomethyl ether which was a liquid and was eventually characterised as its 2:4-dinitrophenylhydrazone.

The positions of the substituent groups in the benzopyrone part of the molecule could be deduced from a combination of demethylation experiments and colour reaction studies. Tetramethyl vogeletin underwent complete demethylation with boiling hydriodic acid to give a pentahydroxyflavone (V). The same compound was obtained from vogeletin by the action of hydriodic acid. This compound (V) which may be called nor-vogeletin was characterised as its penta-acetate and it could be reconverted to tetramethylvogeletin by methylation with dimethyl sulphate and potassium carbonate. Compound (V) answered the Bargellini's test for 5, 6, 7-trihydroxyflavones, but the gossypetone reaction was negative indicating the absence of a 5:8-hydroxy combination. Thus the benzopyrone part in vogeletin, its nor-compound and its tetramethyl ether should carry oxygen functions in positions 5, 6 and 7. The only position now left for the remaining oxygen is position 3 of the benzopyrone ring. Thus tetramethyl vogeletin should be 3, 5, 6, 7, 4'-pentamethoxyflavone (II); nor-vogeletin should be 3, 5, 6, 7, 4'-pentahydroxyflavone (V); and the phenolic ketone should be 2-hydroxy-4, 5, 6-tetramethoxy-acetophenone (III). Structure V for the pentahydroxy compound and II for the complete methyl ether are compatible with the observation that the product of demethylation (VI) of II with anhydrous aluminium chloride is identical with the product of methylation of V with diazomethane; in the former reaction aluminium chloride selectively demethylates the methoxyl in the 5 position, and in the latter reaction diazomethane methylates all the hydroxyls except the one in the 5-position. The melting-points and colour reactions of V and III agree with those described in the literature for compounds having these structures, and the melting-points of II and VI agree with those recorded in the literature. A reference sample of 6-hydroxy-3, 5, 7, 4'-tetramethoxyflavone, kindly supplied by Prof. T. R. Seshadri of the Delhi University, was methylated with dimethyl sulphate and potassium carbonate in acetone solution. The pentamethoxy compound thus obtained and compound II obtained from vogeletin had the same melting-point, both separately and in admixture. Structure III for the phenolic ketone also agrees with our observation that it couples with diazotised sulphanilic acid to yield a coloured dye.
The position of the single methoxyl group (and the four hydroxyl groups) in vogeletin itself could be deduced from the following considerations: When vogeletin was ethylated with diethyl sulphate and potassium carbonate it yielded a tetraethyl ether (VII) which on alkaline fission gave an acid identified as p-ethoxybenzoic acid (VIII). This proves the existence of a free hydroxyl group in position 4' in vogeletin. In contrast to nor-vogeletin (V), vogeletin does not give a positive Bargellini test but it reacts with methylene iodide in the presence of potassium carbonate to give a compound (IX) which contains the methylenedioxy group besides a methoxyl group. This would lead to one of the two alternative combinations, 5-methoxy-6, 7-dihydroxy or 5, 6-dihydroxy-7-methoxy in the benzene ring of the benzopyrone part. Since both dimethyl sulphate and diazomethane react with vogeletin to give the same complete methyl ether (II), position 5 should be already having the methoxyl group and vogeletin should be given the structure 5-methoxy-3, 6, 7, 4'-tetrahydroxyflavone (I). This accords well with its colour reactions. The several transformations mentioned above are represented in the scheme given on page 244.

The occurrence of natural flavonoids carrying a methoxyl group in the 5-position is not very common. There are a few instances where all the hydroxyls including the 5 are methylated, e.g., nobiletin (5, 6, 7, 8, 3', 4'-hexamethoxyflavone), tangeretin (5, 6, 7, 8, 4'-pentamethoxyflavone), meliternatin (3, 5, 7, 8-tetramethoxy-3'-4'-methylenedioxyflavone). But the occurrence of the 5-hydroxy group in the methylated condition, when hydroxyl groups in the more reactive positions occur free, is something extraordinary. Known instances of this type are azaleatin (5-methoxy-3, 7, 3', 4'-tetrahydroxyflavone), muningin (5, 7-dimethoxy-6, 4'-dihydroxyisoflavone) and isopedicin (5, 7, 8-trimethoxy-6-hydroxyflavanone). To explain this it has been suggested by Seshadri that methylation of the concerned hydroxyl group occurs before the pyrone ring closes up and when the reactivity of the specific hydroxyl group is still unhindered.

**Experimental**

The coarsely powdered seeds (20 kg.) were defatted with petroleum ether (4×12 litres). The marc was air-dried, powdered again and extracted with cold methylated spirit (4×12 litres). The alcohol extract was concentrated (to 2 litres) and poured into excess of water (6 litres) and extracted with chloroform to remove the rotenoid compounds and the resins. The clear aqueous liquid having a red colour was concentrated in vacuum (to 2 litres) and the glycoside of the flavone hydrolysed by heating with 7% sulphuric acid. The pigment separated out as a black granular residue.
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which was filtered, washed with water and dried. Crystallization from acetone gave vogeletin as pale yellow needles, m.p. 283-84° (yield 9·0 g.) [Found: C, 61·2; H, 4·3; --OCH_3, 9·5. C_15H_20O_7 requires: C, 60·8; H, 3·8; --OCH_3 (1), 9·8%].

**Vogeletin tetraacetate**

Vogeletin (100 mg.) was boiled with acetic anhydride (2 c.c.) and fused sodium acetate (0·4 g.) for 3 hours in an oil-bath at 145° C. The reaction mixture was poured into water and allowed to stand overnight. The solid was filtered, washed with water and dried. Crystallization from alcohol gave colourless woolly needles, m.p. 211-12° [Found: C, 59·5; H, 4·5; --COCH_3, 34·1; C_24H_20O_11 requires: C, 59·5; H, 4·2; --COCH_3 (4), 35·6%].

**Nor-vogeletin (V)**

Vogeletin (200 mg.) was dissolved in acetic anhydride (5 c.c.) by heating. It was cooled and treated with hydriodic acid (d, 1·7; 10 c.c.). The mixture was heated in an oil-bath at 150-60° for 1 hour. It was then cooled, diluted with water and the solution decolourised by passing sulphur dioxide. The reddish yellow solid was filtered, washed repeatedly with hot water, dried and crystallized from alcohol. The nor-compound is a yellow substance assuming a slight green colour on exposure to air; m.p. is above 320° with darkening and without completely melting. It gives a bluish green ferric colour. Yield 100 mg. (Found: C, 59·7; H, 3·8; --OCH_3, nil. C_{15}H_{16}O_7 requires: C, 59·6; H, 3·3%).

**Nor-vogeletin penta-acetate**

Nor-vogeletin (100 mg.) was acetylated with acetic anhydride (3 c.c.) and fused sodium acetate (350 mg.) in the same way as vogeletin. The acetate crystallized from alcohol as colourless needles, m.p. 228-31° (Found: C, 59·1; H, 4·4. C_{25}H_{20}O_{12} requires: C, 58·6; H, 3·9%).

**O-Tetramethylvogeletin (II)**

(a) *With dimethyl sulphate and potassium carbonate.*—Vogeletin (500 mg.) in acetone (100 c.c.) was treated with dimethyl sulphate (5 c.c.) and anhydrous potassium carbonate (15 g.) and the mixture refluxed on a water-bath for 12 hours. It was filtered, acetone removed from the filtrate and the residue treated with cold water (200 c.c.), stirred well and left in the ice-chest overnight. The solid was filtered, washed with water and dried. It was crystallized from alcohol and then from benzene-petroleum ether, when colourless needles were obtained, m.p. 152-53°. Mixed m.p. with authentic
sample of 3, 5, 6, 7, 4'-pentamethoxyflavone obtained by methylating 3, 5, 7, 4'-tetramethoxy-6-hydroxyflavone with dimethyl sulphate and potassium carbonate was undepressed [Found: C, 64.8; H, 6.0; --OCH₃, 39.7. \( \text{C}_{30}\text{H}_{22}\text{O}_{7} \) requires: C, 64.5; H, 5.4; --OCH₃(5), 41.77%].

(b) With diazomethane.—Vogeletin (100 mg.) was dispersed in absolute methanol (50 c.c.) and treated with excess of diazomethane in ether. The solution assumed a red colour and the substance slowly went into solution. After leaving overnight in the ice-chest, the solution was filtered, the solvents were removed from the filtrate and the residue was crystallized from benzene-petroleum ether. Colourless needles of the methyl ether were obtained, m.p. 152–53°. Mixed m.p. with the product obtained by method (a) was undepressed.

**Methylation of nor-vogeletin**

(a) With dimethyl sulphate and potassium carbonate: Formation of compound II.—Nor-vogeletin (50 mg.) dissolved in acetone (15 c.c.) was methylated with dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (1.5 g.) in the same way as vogeletin. The product obtained (50 mg.) melted at 152–53°, mixed m.p. with the tetramethyl ether of vogeletin was undepressed.

(b) With diazomethane: Formation of compound VI.—Nor-vogeletin (50 mg.) dissolved in absolute methanol (30 c.c.) was methylated by diazomethane as vogeletin. The product crystallized from alcohol as yellow prisms melting at 170–72°. Mixed m.p. with the compound obtained from vogeletin methyl ether (II) by the action of aluminium chloride (described below) was undepressed.

**Demethylation of tetramethylvogeletin**

(a) With hydriodic acid: Formation of compound V.—The methyl ether (200 mg.) dissolved in acetic anhydride (5 c.c.) was demethylated with hydriodic acid \( (d, 1.7; 10 \text{ c.c.}) \) in the same way as vogeletin. Crystallization from alcohol yielded a yellow substance, m.p. above 320° with darkening and without completely melting. Colour reactions were the same as those of the compound obtained from vogeletin by the action of hydriodic acid. The mixed m.p., however, could not be taken since it was too high with charring and incomplete melting.

(b) With anhydrous aluminium chloride: Formation of compound VI.—The methyl ether (100 mg.) in absolute ether (40 c.c.) was treated with anhydrous aluminium chloride (2 g.) and the mixture shaken for ten minutes
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Tetraethyl vogeletin (VII)

Vogeletin (500 mg.) in acetone (100 c.c.) was refluxed for 12 hours with diethyl sulphate (7 c.c.) and anhydrous potassium carbonate (15 g.). After filtering the potassium salts and removing the solvent from the filtrate, a brown red liquid was obtained which remained unchanged on addition of water and leaving in the ice-chest for a day. It was taken into chloroform solution by extracting the mixture with the solvent. The chloroform solution was dried over anhydrous sodium sulphate, filtered and chloroform removed from the filtrate. The brown red liquid remaining was chromatographed on activated alumina (40 g.) employing for elution petroleum ether, benzene, chloroform and mixtures of these in the usual manner. The unchanged diethyl sulphate came down in the first two petroleum ether eluates and the ethyl ether in the subsequent eluates. The fractions showing uniform melting-point were mixed and recrystallized from ether-petroleum ether. The ethyl ether was obtained as colourless prisms, m.p. 84-85 ° (Yield, 450 mg.) [Found: C, 67-8; H, 6.9; --OC--H5, 51-1. C17H12O5 requires: C, 67-3; H, 6-6; --OC--H5(5), 52-6%, all alkoxyls being calculated as ethoxyls].

Methylenation of vogeletin: Formation of compound IX.--Vogeletin (200 mg.), acetone (20 c.c.), anhydrous potassium carbonate (0-5 g.) and methylene iodide (0-15 c.c.) were refluxed on a water-bath for 18 hours. The acetone was removed, water added and left overnight. It was filtered, washed, dried and crystallized from alcohol, when the methylenedioxy derivative (IX) separated as a reddish brown micro-crystalline substance melting at 272-76 ° (Yield, 35 mg.). It gave a green ferri colour, and a green colour on warming with gallic acid in concentrated sulphuric acid [Found: C, 61-7; H, 4.5; --OCH3, 8.6. C17H12O7 requires: C, 62.2; H, 3.7; --OCH3(1), 9.5%].

Alkali fission of tetramethylvogeletin (II)

Tetramethylvogeletin (1 g.) was treated with potassium hydroxide (2.0 g.) in absolute alcohol (40 c.c.) and the mixture refluxed on a water-bath for
6 hours. The solution was cooled, diluted with water and acidified with dilute hydrochloric acid (1:1). The alcohol was removed and the residue extracted with ether. The ether extract was fractionated into sodium bicarbonate-soluble, sodium hydroxide-soluble and neutral fractions.

The acid fraction (400 mg.) crystallized from alcohol as colourless prisms, m.p. 183-84° (IV) (Found: C, 63.6; H, 5.8. Anisic acid requires: C, 63.2; H, 5.3%). Mixed m.p. of IV with authentic sample of anisic acid was undepressed.

The phenolic fraction (560 mg.) was obtained as a reddish brown liquid which solidified after a few days. Crystallization from benzene-petroleum ether gave colourless woolly needles melting at 71-72°. It gave an olive green colour with alcoholic ferric chloride [Found: C, 56.8; H, 6.7; —OCH₃, 46.9. C₁₂H₁₀O₆ (compound III) requires: C, 56.3; H, 6.3; —OCH₃ (4), 48.4%].

The neutral fraction (10 mg.) did not give any crystalline substance.

The 2:4-dinitrophenylhydrazone of the above phenolic ketone (III) prepared in the usual way was obtained as orange woolly needles, m.p. 179-83° (Found: C, 49.7; H, 5.2; —OCH₃, 26.9; N, 12.8. C₁₈H₁₈O₄N₄ requires: C, 49.6; H, 4.6; —OCH₃ (4), 28.4; N, 12.8%).

Alkali fission of tetraethylvogeleatin (VII)

The ethyl ether (350 mg.) was refluxed with an absolute alcoholic potassium hydroxide solution (5%, 20 c.c.) for 6 hours and the products were worked up as in the case of the fission of the methyl ether (II). The acid fraction (VIII) (70 mg.) was recrystallized from alcohol, when it was obtained as colourless prisms, m.p. 194-95° (Found: C, 65.4; H, 6.5. p-Ethoxy benzoic acid requires: C, 65.0; H, 6.1%). Mixed m.p. of the above product (VIII) with p-ethoxybenzoic acid prepared according to the method of Cohen and Dudley was undepressed. The phenolic fraction was a thick red liquid which did not solidify and was not examined further,
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SUMMARY

The constitution of the pigment isolated from the seeds of Tephrosia vogelii Hook. (now designated as vogeletin) has been established, by a study of its derivatives and by chemical degradation, as 5-methoxy-3, 6, 7, 4'-tetrahydroxyflavone.

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REFERENCES