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ISOLATION OF (+) O-PENTAMETHYL DIHYDRO-MELANOXETIN FROM *ALBIZZIA ODORATISSIMA* BENTH.

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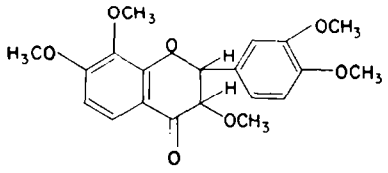
Abstract—From the heartwood of *A. odoratissima* Benth. a dihydroflavonol was isolated as its methyl ether. From its degradation reactions, it was shown to be (+) O-pentamethyl dihydromelanoxetin. The synthesis of a racemate of this constitution is also reported.

THE *Albizzia* heartwoods[1] do not form commercially important timber, but are used extensively for minor constructions. Under a general scheme, the heartwoods of *A. amara*, *A. odoratissima*, *A. procera* and *A. lebbek* were examined but they are singularly free from triterpenoids, although the nuts and barks of *A. procera*[2], *A. lebbek*[3] and *A. odoratissima*[4] are reported to contain triterpenoid saponins of echinosystic and albigenic acids. Machaeric acid was also reported from the bark of *A. procera*.[2]

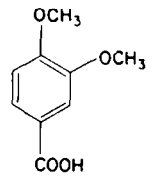
The heartwood of *A. odoratissima* has been examined. The ether and petroleum ether extracts yield two neutral compounds, A and B, (m.p. 88–89° and 144–145°). From the acetone extract, the ethyl acetate soluble fraction gives positive tests for leucoanthocyanidins; but no crystalline compound could be separated even by the counter-current distribution method of Bush and Denson[5] between water and ethyl acetate. The gummy solid was, therefore, completely methylated using diazomethane. The product, m.p. 146–148° $(\alpha)_D^{30°}$ +88°; analysed for $C_{20}H_{22}O_7$ with five methoxyls. It does not exhibit the pink colouration with butanol–HCl characteristic of a leucoanthocyanidin; but its flavonoid character was established beyond doubt by the pink colouration with Zn and HCl[6] and bright reddish violet colour when treated with magnesium and HCl. Its U.V. absorption spectrum[7] (λ_{max} 248, 346 mμ, log ϵ 3·6 and 3·62) suggests that it could be a dihydroflavonol; but the I.R. spectrum is singularly free from any hydroxyl absorption; intense absorption at 6·16 μ (1623 cm^{-1}) suggests the presence of a pyrone carbonyl[8] in the molecule.

During both alkaline hydrolysis and permanganate oxidation reactions, veratric acid (II) and 2-hydroxy-3,4-dimethoxybenzoic acid (III) are produced. These acids were also formed during oxidation with alkaline hydrogen peroxide, but mild oxidation with potassium acetate–iodine yields O-pentamethylmelanoxetin (IV). The new compound from *A. odoratissima* should therefore be (+) O-pentamethyldihydromelanoxetin (I).

[1] R. S. Pearson and H. P. Brown, *Commercial Timbers of India* Vol. I, 461. Govt. of India, Calcutta 1932.
[2] M. D. Farooq, I. P. Varshney and H. Hassan, *Curr. Sci.* **47**, 489 (1958).
[3] A. K. Barua and S. P. Raman, *Tetrahedron* **7**, 19 (1959).
[4] I. P. Varshney and M. S. Y. Khan, *J. Sci. Ind. Res.* **21B**, 30 (1962).
[5] T. Bush and M. Denson, *Analyt. Chem.* **20**, 121 (1948).
[6] J. C. Pew, *J. Amer. Chem. Soc.* **70**, 3031 (1948).
[7] H. Erdtman, *Svensk Kem. Tid.* and G. Linstead, *Acta. Chem. Scand.* **4**, 772 (1950).
[8] H. L. Hergert and E. F. Kurth, *J. Amer. Chem. Soc.* **75**, 1622 (1953).



I



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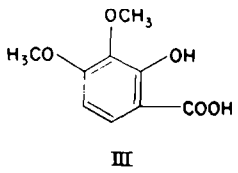

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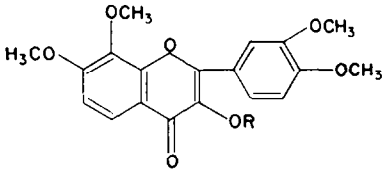
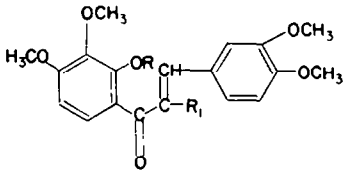
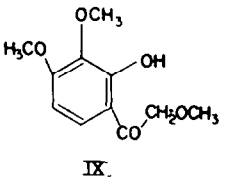


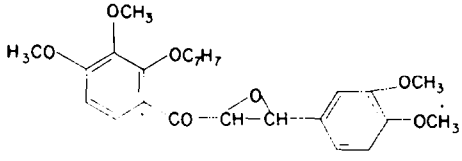
IV R=CH_3
V R=H



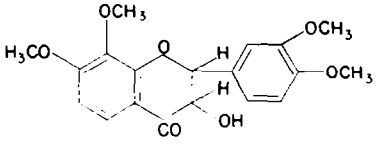
VI R=C_7H_7; R_1=H
X R=H; R_1=OCH_3



IX



VII



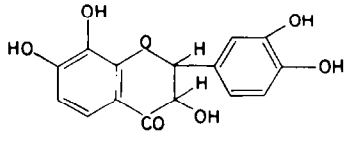
VIII

This structure was further confirmed by the identification of 3-hydroxy-7,8,3′,4′-tetramethoxyflavone (V) among the products of alkaline hydrolysis. The formation of a flavonol from a dihydroflavonol during alkaline hydrolysis has already been reported: for example izalpinin from alpinone,[9] O-pentamethyl myricetin from ampelopsin pentamethyl ether.[10] It is obvious that the dihydromelanoxetin pentamethyl ether (I) undergoes demethylation at the third position followed by a ready dehydrogenation in the presence of alkali and air.[11]

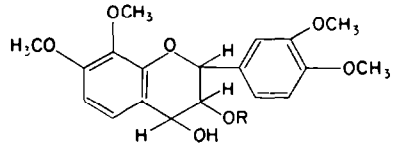
The constitution of O-pentamethyldihydromelanoxetin was confirmed by its synthesis following the method of Bognar and Stefarovsky.[12] The epoxide (VII) of 3,4,3′,4′-tetramethoxy-2′-benzyloxychalkone (VI) is better prepared by the action of hydrogen peroxide at 0° than at higher temperatures. The epoxide cyclizes to 3-hydroxy-7,8,3′,4′-tetramethoxyflavanone (VIII). It yields a methyl ether as a racemate, the melting point of which is not depressed by O-pentamethyldihydromelanoxetin. The 3-hydroxyflavanone (VIII) was also synthesized following the method of Joshi and Kulkarni[13] by the action of aqueous sodium carbonate on the acetate of the corresponding chalkone dibromide. But in this reaction, the dihydroflavonol (VIII) is always accompanied by the corresponding flavone (V).

[9] Yushiro Kimura and Morio Hoshi, *Proc. Imp. Acad. Tokyo* **12**, 285, (1936).
[10] Munio Kotake and Takashi Kuboto, *Liebig Ann.* **544**, 253, (1940).
[11] T. R. Seshadri, *Tetrahedron* **6**, 181, (1959).
[12] R. Bognar and J. Stefarovsky, *Tetrahedron* **18**, 143, (1962).
[13] A. B. Kulkarni and C. G. Joshi, *Chem. & Ind.* 1456, (1954).

O-Pentamethyldihydromelanoxetin (I) during alkaline hydrolysis for 30 minutes in an inert atmosphere yields ω-3,4-trimethoxy-2-hydroxyacetophenone (IX) in addition to the two acids (II and III) instead of the chalkone (X), as in alpinone[9] and ampelopsin.[10] The chalkone (X) was, however, successfully obtained in low yield, when hydrolysis was carried out with 15 per cent alkali for 5 minutes. The formation of the ω-methoxyketone (IX) from O-pentamethyldihydromelanoxetin (I) is unique as is its behaviour in the presence of alkali giving rise to all the possible degradation products. Further, the dihydromelanoxetin (XI) which could not be isolated, was completely methylated even by diazomethane. This ease of methylation of the 3-hydroxyl in dihyromelanoxetin has not been recorded previously.



XI



XII, R=CH_3
XIII R=H

The reduction of (+) O-pentamethyldihydromelanoxetin (I) either with Pt-H or with $NaBH_4$ yields a leucoanthocyanidin pentamethyl ether (XII) with diminished optical rotation $(\alpha)_D^{30°}$ +8·6°. Attempts to convert the latter into melacacidin tetramethyl ether (XIII) by controlled hydrolysis were unsuccessful; in every case the anthocyanidin being formed.

O-Pentamethyldihydromelanoxetin (I) undergoes demethylation with hydroiodic acid and a little red phosphorus to give a pentahydroxy derivative (XI) $(\alpha)_D^{30°}$ +101·3°, which may be remethylated to a pentamethyl ether (I) having a higher melting point 152° and a higher optical rotation, $(\alpha)_D^{30°}$ +120·3°. This is substantially different from the original compound (I) as indicated by the I.R. spectrum. The nature of this isomeric change is not clear; but it was also noticed during demethylation with aluminium chloride.

The isolation of O-pentamethyl ether of XI from the heartwood of *A. odoratissima* is the first recorded occurrence of dihydromelanoxetin in Nature. The corresponding leucoanthcyanidin, (−) melacacidin, has been isolated from *Acacia melanoxylon*[14] and other *Acacia* species.[15]

EXPERIMENTAL

Powdered heartwood (2 Kg) of *Albizzia odoratissima* was successively extracted with petroleum ether (16 hr), ether (48 hr) and acetone (30 hr) in a soxhlet extractor.

Petroleum ether extract. The light yellow extract (4 l.) on concentration (250 ml) deposited a pale yellow solid (500 mg) (compound A) which crystallized from benzene–pet ether as colourless prisms, m.p. 85–86°, (Found: C, 73·3; H, 11·3%). The mother liquors on further concentration gave another compound (300 mg; compound B) which crystallized from pet ether as white shining needles, m.p. 145–46° (Found: C, 83·0; H, 11·78%). *Ether extract.* During extraction a brown solid separated, which on crystallization from acetone gave prismatic needles, m.p. 85–87°, undepressed by compound (A), yield (8 g).

Acetone extract. The dark brown viscous residue (250 g) left after the removal of the solvent under vacuum, was stirred with water (1·5 l.) for 1 hr and left over night. The clear filtrate was decanted and concentrated under red. press. at 40–50° to 300 ml. It was shaken with ethyl acetate

[14] F. E. King and W. Bottomley, *J. Chem. Soc.* 1399, (1954).
[15] J. W. Clark Lewis and P. I. Mortimer, *J. Chem. Soc.* 4108, (1960).

(6 × 100 ml) and the organic layer evaporated to a brown gummy mass (20 g). It was redissolved in water (100 ml) and distributed between ethyl acetate (50 ml) and water following the counter current distribution method of Bush and Densen[5] with five separatory funnels. Evaporation of all the ethyl acetate phases gave only light brown semi solids. For purification, these fractions were combined and dissolved in ethyl acetate. Careful addition of light pet ether (40–60°) caused precipitation of coloured impurities and finally dilution with pet ether (4 vol) deposited a light brown gum (10 g); $FeCl_3$ colour green to blue; crimson colour with alcoholic HCl. As it did not crystallize either from acetone–pet ether or from any other solvent, the product in methanol was treated with excess diazomethane in ether. After 24 hr at 0°, removal of the ether furnished a gum which after two crystallizations from ethyl acetate–pet ether gave O-pentamethyldihydromelanoxetin, as pale yellow shining needles, m.p. 146–48° yield 1·5 g; $(\alpha)_D^{30°}$ +88° (*c*, 0·625 in ethanol). A sample dried at 100° for 6 hr at 0·4 mm was analysed. (Found: C, 64·37; H, 6·05; OMe, 40·0; $C_{20}H_{22}O_7$ requires: C, 64·16; H, 5·88; 5-OMe, 41·40%). U.V.: λ_{max} 346, 248 mμ, log ϵ 3·6, 3·62; I.R.: λ 6·16 μ (1623 cm^{-1}, pyrone C=O).

The methyl ether exhibited a pale yellow colour with alcoholic HCl, a deep reddish pink colour with Mg–HCl and pink colour with Zn–HCl.

Methylation of the gum in acetone with K_2CO_3–Me_2SO_4 also yielded the same methyl ether.

Oxidation of O-*pentamethyl dihydromelanoxetin*

(a) *With* $KMnO_4$. The methyl ether (100 mg) in acetone (20 ml) was refluxed with $KMnO_4$ (150 mg) for 4 hr the acetone removed, water added and the solution saturated with sulphur dioxide. The clear solution was thoroughly extracted with ether and the ether extract repeatedly washed with 4% sodium bicarbonate solution (3 × 15 ml). The bicarbonate layer was neutralized with HCl and thoroughly extracted with ether. The resulting extract was treated with an ethereal solution of diazomethane. After 24 hr excess diazomethane was removed and the residual ethereal layer washed with cold 4% sodium hydroxide (3 × 10 ml). The alkaline layer was run into ice-cold conc. HCl with separation of the methyl ester of an O-hydroxycarboxylic acid. It crystallized from aqueous methanol as white shining needles, m.p. 78–80° (40 mg) undepressed by a synthetic sample of methyl-2-hydroxy-3,4-dimethoxybenzoate. (Found: C, 56·03; H, 5·92; OMe, 43·15; $C_{10}H_{12}O_5$ requires C, 56·61; H, 5·66; 3-OMe, 43·8%).

The methyl ester was refluxed with 10% aqueous KOH for 30 min and acidified. The resulting O-hydroxybenzoic acid crystallized as white shining needles from aqueous methanol, m.p. 170–172° undepressed by a synthetic sample of 2-hydroxy-3,4-dimethoxybenzoic acid. (Found: C, 54·7; H, 5·66; OMe, 30·72; $C_9H_{10}O_5$ requires C, 54·54; H, 5·05; 2-OMe, 31·3%).

The methyl ester as well as the acid exhibited a bluish violet colour with alcoholic ferric chloride.

After removal of the phenolic methyl ester (see above), the ethereal layer was evaporated to give a low melting solid, m.p. 54–56°. On alkaline hydrolysis, it gave rise to veratric acid, yield (23 mg).

(b) *With alkaline hydrogen peroxide.* To a solution of the methyl ether (100 mg) in 10% alcoholic NaOH (10 ml) at 10–15°, hydrogen peroxide (30 vols, 5 ml) was added dropwise till a permanent pale yellow colour was obtained. After leaving it overnight at room temp, the solvent was removed and dilute HCl added. A slight precipitate was formed. It was extracted with ether and then separated into an acidic fraction (70 mg) and a negligible amount of neutral gummy residue. The acidic fraction ($FeCl_3$: bluish violet) was separated into veratric acid (40 mg) and 2-hydroxy-3,4-dimethoxybenzoic acid (35 mg) as before.

Conversion of O-*pentamethyl dihydromelanoxetin to* O-*pentamethyl melanoxetin.*

The methyl ether (50 mg) in acetic acid (4 ml), fused potassium acetate (300 mg) and iodine (40 mg) were refluxed for 2 hr. Acetic acid was removed under vacuum, water added and excess iodine removed by saturation with sulphur dioxide. The product crystallized from ethanol as white shining needles m.p. 148–150°, undepressed by 3,7,8,3′:4′-pentamethoxyflavone. $(\alpha)_D^{30°}$ nil. yield: 30 mg. (Found: C, 64·45; H, 5·02; OMe, 41·4; $C_{20}H_{20}O_7$ requires C, 64·52; H, 5·37; 5-OMe, 41·67%).

Synthesis of 2-*hydroxy*-3,4-*dimethoxybenzoic acid*

According to the method of Sarin and Seshadri[16] 2-hydroxy-3,4-dimethoxyacetophenone (500 mg) was dissolved in pyridine (16 ml) and iodine (2·5 g) added. The dark coloured solution was heated for 1 hr on a steam bath and then kept at 0° for 24 hr. Pyridine was removed under vacuum and the

[16] P. S. Sarin and T. R. Seshadri, *Tetrahedron* **8**, 65 (1960).

iodine complex decomposed by heating with 2% KOH (25 ml) for 2 hr. on a steam bath. It was acidified with HCl and excess iodine destroyed by sulphur dioxide. 2-Hydroxy-3,4-dimethoxybenzoic acid was crystallized from aqueous alcohol as colourless needles; m.p. 170–172°, yield, 280 mg, $FeCl_3$ colour: bluish violet.

The methyl ester (diazomethane) crystallized from aqueous methanol as shining colourless needles, m.p. 78–80°; $FeCl_3$ colour: violet.

Hydrolysis of O-*pentamethyldihydromelanoxetin*

(a) The methyl ether (100 mg) was refluxed with 8% absolute alcoholic KOH (10 ml) for ½ hr in a nitrogen atmosphere. Alcohol was removed under vacuum, water (15 ml) added and the aqueous alkaline solution washed with ether to remove the unreacted compound. The alkaline solution was neutralized with HCl and thoroughly extracted with ether. The ether layer was washed with 4% sodium bicarbonate and then with 4% sodium hydroxide solution. The bicarbonate layer on neutralization gave 2-hydroxy-3,4-dimethoxybenzoic acid (20 mg) and veratric acid (30 mg).

The alkaline extract gave on neutralization a colourless compound. It crystallized from pet ether as colourless prismatic needles; m.p. 82–84° undepressed by 2-hydroxy-3,4-ω-trimethoxyacetophenone obtained from O-pentamethylmelanoxetin by alkaline hydrolysis. (Found: C, 58·72; H, 6·49; OMe, 40·45; $C_{11}H_{14}O_5$ requires: C, 58·41; H, 6·19; 3-OMe, 41·15%).

(b) The methyl ether (100 mg) was refluxed with 8% alcoholic potash for 2 hr; the alcohol evaporated and diluted with water (20 ml). The product as in (a) was separated into acidic and phenolic components. The acidic mixture was further separated as before and the components identified as veratric acid (20 mg) and 2-hydroxy-3,4-dimethoxybenzoic acid (20 mg). The phenolic fraction was crystallized from aqueous methanol as yellow prisms (20 mg) m.p. 218–220° unchanged by an authentic sample of 3-hydroxy-7,8,3′:4′-tetramethoxyflavone.

(c) The methyl ether (100 mg) was heated under reflux with 15% absolute alcoholic KOH for 5 min and neutralized with HCl. The bicarbonate insoluble fraction of the resulting precipitate was crystallized from benzene–pet ether as light yellow impure plates, m.p. 75–77°; yield: 10 mg. It exhibited all the reactions of a chalkone; NaOH–bright red; Mg–HCl—no colour.

Reduction of O-*pentamethyldihydromelanoxetin*

(a) The methyl ether (100 mg) in acetic acid (15 ml) was hydrogenated using Adam's catalyst. The reduced product crystallized from aqueous ethanol as white shining needles (50 mg) m.p. 140–141°, $(\alpha)_D^{30°}$ +8·6° (*c*, 1·05 in ethanol). The mixed m.p. with the parent compound was depressed to 120°. It developed a crimson colour on warming with HCl in butanol. (Found: C, 61·95; H, 6·70; OMe, 39·35; $C_{20}H_{24}O_7$, ½ H_2O requires: C, 62·24; H, 6·49; 5-OMe, 40·25%).

(b) The methyl ether (100 mg) in aldehyde free ethanol (20 ml) was treated with sodium borohydride (50 mg) and after 24 hr alcohol was removed under red press. and the residue dissolved in water (8 ml). The solution was acidified with acetic acid and extracted with ether. The ether layer on evaporation gave a white solid (50 mg), crystallized from ethanol as white needles; m.p. 140–142° undepressed by the compound obtained in (a).

Demethylation of O-*pentamethyldihydromelanoxetin*

(a) The methyl ether (100 mg) in anhydrous chlorobenzene (6 ml) was heated at 100° with anhydrous aluminium chloride (150 mg) for 15 min. The excess reagent was decomposed with ice cold HCl (5 ml) and the chlorobenzene removed by steam distillation. The solid residue was separated into a phenolic and a neutral product. The phenolic component cyrstallized from aqueous ethanol as yellow shining needles (15 mg) m.p. 140–142°, $(\alpha)_D^{30°}$ +122·5° (*c*, 0·089 in ethanol), $FeCl_3$ colour—brown; Mg–HCl—pink colour. I.R. λ 3·0 μ (3335 cm^{-1} for OH); 6·10 μ (1639 cm^{-1} for pyrone C=O). Methyl ether of this compound (diazomethane) crystallized as white shining needles from aqueous ethanol, m.p. 150–152°, unchanged by O-pentamethyldihydromelanoxetin. $(\alpha)_D^{28°}$ +110° (*c*, 0·127 in ethanol); $FeCl_3$ colour—nil. I.R. λ 6·1 μ (1639 cm^{-1} for pyrone C=O).

The neutral compound gave white shining needles, m.p. 150–152°; $(\alpha)_D^{28°}$ +110° (*c*, 0·1089 in ethanol) undepressed by the above methyl ether (Found: OMe, 41·62; $C_{20}H_{22}O_7$ for 5-OMe requires: 41·44%).

(b) The methyl ether (100 mg) in acetic anhydride (3 ml) was refluxed with hydriodic acid (M.A.R. 6 ml) for 2 hr in presence of red phosphorous (50 mg). It was cooled, filtered, diluted with

water (10 ml) and decolourized by passing sulphur dioxide. The resulting product crystallized from ethanol as yellow amorphous powder (50 mg) m.p 300–305° (dec.); $(\alpha)_D^{30°}$ +101·3° (*c*, 0·236 in ethanol). (Found: C, 55·94; H, 4·05; $C_{18}H_{12}O_7$, H_2O requires: C, 55·91; H, 4·34%). Mg–HCl—pink; Zn–HCl—pinkish red colour.

Remethylation of this hydroxy compound with diazomethane gave colourless needles, m.p. 150–152°, unchanged by O-pentamethyldihydromelanoxetin, $(\alpha)_D^{25°}$ +120·3° (*c*, 0·232 in ethanol) (Found: C, 64·48; H, 5·98; OMe, 41·2; $C_{20}H_{22}O_7$ requires: C, 64·16; H, 5·88; 5-OMe, 41·4%).

Synthesis of 7,8,3′,4′-*tetramethoxydihydroflavonol*

(a) *Epoxide of* 2′-*benzyloxy*-3,4,3′,4′-*tetramethoxychalkone.* The benzylation of 2′-hydroxy-3,4,3′,4′-tetramethoxychalkone was carried out with acetone–K_2CO_3–benzyl chloride. The benzyl ether (yield: 80%) crystallized from methanol as light yellow needles m.p. 96–98°. (Found: C, 71·4; H, 5·18; OMe, 28·43; $C_{26}H_{26}O_6$ requires: C, 71·9; H, 5·991; 4-OMe, 28·57%).

The benzyloxychalkone (250 mg) was treated with alkaline hydrogen peroxide (0·3 ml) in acetone (10 ml) at 0° and left for 24 hr. The epoxide (200 mg, yield 90%) crystallized in needles from methanol, m.p. 120–121°. (Found: C, 68·9; H, 5·52; OMe, 27·4; $C_{26}H_{26}O_7$ requires: C, 69·18 H, 5·77; 4-OMe, 27·56%).

(b) O-7,8,3′,4′-*Tetramethoxydihydroflavonol.* An ice cold ethereal solution of the above epoxide was saturated with dry HCl gas. After removal of the solvent, the dihydroflavonol was crystallized from acetone as white prisms, m.p. 160–162°, insoluble in aqueous alkali and showing only a faint brown colour with ferric chloride; a pink colour with Zn–HCl and a deep red colour with Mg–HCl. (Found: C, 63·25; H, 5·4; OMe, 33·9; $C_{19}H_{20}O_7$ requires: C, 63·3; H, 5·5; 4-OMe, 34·63%).

Methylation of O-7,8,3′,4′-*tetramethoxydihydroflavonol*

The dihydroflavonol (100 mg) in acetone (10 ml) was refluxed with dimethyl sulphate (0·5 ml) over K_2CO_3 (150 mg) for 6 hr. The solution was filtered, the acetone evaporated and the residue treated with 8% ammonium hydroxide (10 ml) to decompose the excess dimethyl sulphate yielding a white solid which crystallized from ethanol as white needles (20 mg), m.p. 145–148° undepressed by the O-pentamethyldihydromelanoxetin, $FeCl_3$ colour—nil; conc. H_2SO_4 colour—yellow; Zn–HCl colour—pink. (Found: C, 64·37; H, 5·53; OMe, 41·0; $C_{20}H_{22}O_7$ requires: C, 64·16; H, 5·88; 5-OMe, 41·4%).

(b) The dihydroflavonol (200 mg) was refluxed with sodium methoxide and methyl iodide (2.5 ml) for 40 hr. On cooling a yellow solid separated and was crystallized from methanol as yellow needles (50 mg) m.p. 195–197°; orange red colour with conc. H_2SO_4; $FeCl_3$ colour—nil; Mg–HCl or Zn–HCl—nil. (Found: C, 66·92; H, 5·77; OMe, 34·61; $C_{19}H_{18}O_6$ requires: C, 66·67; H, 5·264; 4-OMe, 36·2%).

Acknowledgements—One of us (C.V.R.S.) wishes to express his grateful thanks to The Council of Scientific and Industrial Research, Delhi, for a fellowship. Our thanks are also due to Profs. J. W. Clark Lewis, T. R. Seshadri and N. V. Subbarao for the I.R. spectra reported. Our thanks are also due to Prof. J. W. Clark Lewis for a sample of melacacidin tetra methyl ether.