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NEW COMPONENTS OF *CITRUS AURANTIUM*

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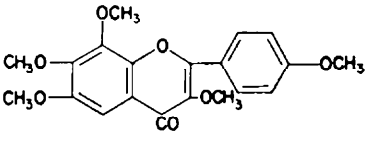
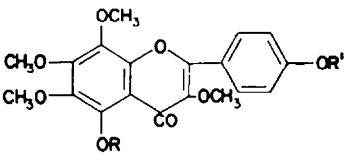
Abstract—Further investigations of the peel of *Citrus aurantium* (from Waltair) have yielded a new flavonoid 5-hydroxyauranetin. 5-O-desmethylnobiletin has been isolated from the peel of Nagpur oranges along with auranetin and hesperidin. These results are of biogenetic significance.

PREVIOUS investigations[1,2] of the peel of Kamala oranges have revealed the presence of auranetin, 3,6,7,8,4′-pentamethoxyflavone (I). Re-investigation has revealed the presence of a new flavonoid shown to be 5-hydroxyauranetin (III). On methylation this gives calycopterin dimethyl ether (5-methoxyauranetin II), which on fission with alkali gives anisic acid and 2-hydroxy-ω-3,4,5,6-penta methoxy acetophenone. The m.p. of III agrees with that of 4′-0-methylcalycopterin (III),[3,4] and the identification was further confirmed by comparison with authentic samples prepared from calycopterin (IV) isolated from the leaves of *Calycopterus floribunda*.[5,6]

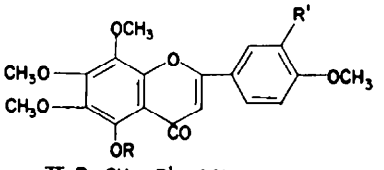


I

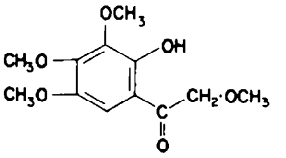


II, R = R′ = CH_3
III, R = H, R′ = CH_3
IV, R = R′ = H

The peel of Nagpur oranges yields auranetin and desmethylnobiletin (VI) which contains one hydroxyl group and five methoxy groups and is isomeric with 5-hydroxy-auranetin. On methylation VI gives nobiletin (V), and on demethylation it gives nornobiletin.[7,8] Partial methylation of the latter gives desmethylnobiletin.[7]



V, R = CH_3, R′ = OCH_3
VI, R = H, R′ = OCH_3
X, R = CH_3, R′ = H



VII

The occurrence of I and III in the peel of *Citrus aurantium* (Waltair variety) is significant. The suggestion was made[2] that the biogenisis of I should closely follow

[1] K. C. Patnayak, S. Rangaswami and T. R. Seshadri, *Proc. Indian Acad. Sci.* **16A**, 10 (1942).
[2] V. V. S. Murti, S. Rangaswami and T. R. Seshadri, *Proc. Indian Acad. Sci.* **28A**, 19 (1948).
[3] R. C. Shah, V. V. Virkar and K. Venkataraman, *J. Indian Chem. Soc.* 135 (1942).
[4] T. R. Seshadri and N. Viswanadham, *Proc. Indian Acad. Sci.* **25A**, 337 (1947).
[5] A. N. Ratnagiriswaran, K. B. Sehra and K. Venkataraman, *Biochem. J.* **28**, 1964 (1934).
[6] T. R. Seshadri and V. Venkateswarlu, *Proc. Indian Acad. Sci.* **23A**, 192 (1946).
[7] R. Robinson and K. F. Tseng, *J. Chem. Soc.* 1004 (1938).
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that of the related flavonols having substituents in the 5,6,7,8,-positions and belonging to the calycopterin series. The removal of the 5-hydroxyl group in the flavone system has been shown[9] and the nuclear reduction of III has now been achieved by preparing the tosyl derivative which is readily reduced with Raney Nickel to yield auranetin.

The occurrence of V in *Citrus nobilis*[10] and VI with auranetin in *Citrus aurantium* is also significant. It supports the above-mentioned biogenetic relationship and indicates that the final methylation of a resistant 5-hydroxyl group can take place in the plant.

The ketone from alkali fission of auranetin has the constitution VII which has now been established by oxidation[11] to 2-hydroxy-3,4,5-trimethoxybenzoic acid.[12]

Tangeretin, a fully methylated flavone occurring in *Citrus nobilis deliciosa*[13] is a lower member of the nobiletin series (X), and ponkanetin[14] isolated from *Citrus poonensis* Hort is identical with it.[15,16]

EXPERIMENTAL

Peel of the Waltair variety of Citrus aurantium

Extraction. The air dried peel (2 kg) was extracted twice with light petroleum (b.p. 60–80°) for 24 hr in the cold to remove essential oils and fatty and waxy material. The extraction was repeated thrice with more light petroleum by refluxing for 10 hr each time. The hot extract after concentration deposited a pale yellow solid which was filtered, taken up in ether and the ether solution extracted with 5% NaOH (fraction A). Evaporation of the remaining ether solution yielded a neutral fraction B.

The residual peel was extracted with boiling alcohol for 10 hr. The alcoholic extract was evaporated under reduced pressure to a reddish brown concentrate. This was extracted with ether leaving an insoluble residue (fraction C). The etheral solution extracted with 5% NaOH aq. again yielded fraction A, and the residual ether solution yielded fraction B.

Fraction A (*5-hydroxy auranetin*, III). Acidification yielded a yellow solid, which crystallized from alcohol as yellow prisms, m.p. 125–27°. In alcoholic solution it gave a red colour with magnesium or zinc and hydrochloric acid and an olive green colour with ferric chloride (Found: C, 61·9; H, 5·3, OCH_3, 39·0, Calc. for $C_{20}H_{20}O_8$: C, 61·9; H, 5·2; OCH_3, 40%). Yield, 0·25 g. Mixed m.p. with an authentic sample of 5-hydroxy auranetin (4′-O-methyl calycopterin[4]) was undepressed.

Methylation of A *to* (*5-methoxy auranetin* II). The above compound (0·1 g) was refluxed with acetone (100 cc), dimethyl sulphate (2 cc), and potassium carbonate (5 g) for 40 hr. The methyl ether crystallized from ethyl acetate–light petroleum mixture as colourless plates, m.p. 132–34° (Found: C, 62·4; H, 5·6; Calc. for $C_{21}H_{22}O_8$: C, 62·7; H, 5·5%). Mixed m.p. with a synthetic sample of calycopterin dimethyl ether was undepressed. The infra-red spectrum showed peaks at (microns) 6·1 (S), 6·2 (S), 6·3 (W), 6·7, 7·1 (S), 7·9 (S) 8·5 (S), 8·8 (W), 9·3, 9·5 (S), 11·9 (S), 12·7 (W).

Fission of methyl ether (II) *to 2-hydroxy-ω-3:4:5:6-pentamethoxy-acetophenone.* The methyl ether (0·05 g) was boiled under reflux with 8% absolute alcoholic potash for 6 hr. The sodium bicarbonate soluble fraction yielded anisic acid, m.p. and mixed m.p. 182–84°. The ketonic product gave a green colour with ferric chloride. It was isolated as the 2:4-dinitro-phenylhydrazone, m.p. 173–75°.[8]

Tosylation of A. The hydroxy compound (0·13 g) in acetone (100 cc) was refluxed with *p*-toluene sulphonyl chloride (0·3 g) and potassium carbonate (10 g) on a water bath until the product gave no colour with alcoholic ferric chloride (16 hr). The tosyl ester crystallized from alcohol as colourless prisms, m.p. 165–67° (Found: C, 60·2; H, 4·6; $C_{27}H_{26}SO_{10}$ requires: C, 59·8; H, 4·8%).

Raney nickel reduction. The tosyl ester (0·07 g) was dissolved in alcohol (300 cc) and Raney

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[10] K. F. Tseng, *J. Chem. Soc.* 1003 (1938).
[11] N. A. Lund, A. Robertson and W. B. Whalley, *J. Chem. Soc.* 2439 (1953).
[12] A. M. Hamburg, *Monatsh* **19**, 593 (1898).
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[16] Matsuura, *J. Pharm. Soc., Japan* 77, 702 (1957).
[17] V. V. S. Murti, K. V. Rao and T. R. Seshadri, *Proc. Indian Acad. Sci.* **24A**, 233 (1946).

nickel[18] (two teaspoonfuls) added. A stream of purified hydrogen was passed for 1 hr with vigorous shaking. The alkali insoluble product crystallized from alcohol as colourless needles, m.p. 139–40°. Mixed m.p. with an authentic sample of auranetin was undepressed. Comparison of the infra-red spectra of the nuclear reduction product and auranetin confirmed identity.

Fraction B (*auranetin* I). Complete evaporation of the ether solution left a pale yellow solid which crystallized from alcohol as colourless needles, m.p. 139–40°. It gave no colour with alcoholic ferric chloride. Yield 1·0 g (Found: C, 64·8; H, 5·7; Calc. for $C_{20}H_{20}O_7$: C, 64·5; H, 5·4%). The infra-red spectrum showed the following peaks at (microns) 6·1 (S), 6·7 (W), 7·3 (S), 7·7 (W), 7·8 (S), 9·3 (S), 9·8 (S), 10·3 (W), 11·9, 12·3 (W).

Demethylation of fraction B using hydriodic acid yielded a product which was identical with norauranetin in all respects.

Fission of auranetin to 2-hydroxy-ω:3:4:5-tetramethoxy-acetophenone (VII). (0·75 g) in absolute alcoholic potash (2 g in 25 cc) was refluxed on a water bath. The bicarbonate soluble fraction gave anisic acid, m.p. and mixed m.p. 182–84°. The potash soluble fraction (hydroxy-ketone) crystallized from alcohol as pale yellow prisms, m.p. 116–17°. It gave a brown colour with alcoholic ferric chloride (Found: C, 56·5; H, 6·5, OCH_3, 48·6; $C_{12}H_{16}O_6$ requires: C, 56·2; H, 6·3, OCH_3, 48·5%).

Oxidation of the ketone to 2-hydroxy-3:4:5-trimethoxy benzoic acid (VIII). A mixture of the above ketone (0·2 g), iodine (0·8 g) and pyridine (5 cc) was heated in a steam bath for 1 hr and then kept at 0° for 24 hr. The alcoholic solution of the pyridinium iodide complex was heated with aqueous potash (2%, 25 cc) in a steam bath for 1 hr. The acid product crystallized from ethyl acetate–light petroleum mixture as colourless needles, m.p. 190–92° (decomp). Mixed m.p. with an authentic sample of 2-hydroxy-3:4:5-trimethoxy benzoic acid[12] was undepressed.

The above method of oxidation had not been used with ω-methoxy acetophenones. Hence ω-methoxy phloroacetophenone dimethyl ether was used for test experiment under identical conditions and it gave 4:6-dimethoxy salicylic acid, m.p. 152–54° (decomp); Josephson[19] also reported the same m.p. for the sample obtained by a different method.

Fraction C (*hesperidin*). This was sparingly soluble in most solvents and was crystallized from pyridine; the colourless crystalline solid melted at 250–51° (2·5 g). Its reactions agreed with those of hesperidin and the mixed m.p. with an authentic sample was undepressed.

Peel of Nagpur variety

The extraction of the air dried peel was repeated. The petroleum ether extract gave a 5% NaOH soluble fraction A′ and a neutral fraction B′. The alcoholic extract gave in addition to the above two components, a glycosidic fraction C′.

Fraction A′ (5-O-*desmethyl nobiletin* VI). This fraction crystallized from alcohol as colourless needles, m.p. 144–46° (0·5 g). It gave a deep red colour with magnesium and alcoholic hydrochloric acid and a green colour with alcoholic ferric chloride (Found: C, 61·5; H, 5·6; OCH_3, 40·8; Calc. for $C_{20}H_{20}O_8$: C, 61·9; H, 5·2; OCH_3, 40%). Robinson and Tseng[7] reported the same m.p.

Methyl ether of A′ (*nobiletin* V). The methylation of the above compound was effected with dimethyl sulphate using acetone and potassium carbonate by refluxing for 54 hr. The product crystallized from ethyl acetate as pale yellow prisms, m.p. 129–31°. It gave no colour with alcoholic ferric chloride. (Found: C, 62·5; H, 5·7; Calc. for $C_{21}H_{22}O_8$: C, 62·7; H, 5·5%).

The fission of the methyl ether (0·1 g) was effected by refluxing with alcoholic potash (20 cc, 20%) for 4 hr. The sodium bicarbonate soluble fraction gave veratric acid, m.p. and mixed m.p. 179–80°.

Demethylation of A′ *to nornobiletin.* Compound A′ (0·2 g) was boiled with hydriodic acid (8 cc) and acetic anhydride (20 cc). The hydroxy flavone so obtained crystallized from absolute alcohol as yellow prisms, m.p. 310–12° (decomp). It gave an olive green colour with alcoholic ferric chloride and agreed in chromatographic behaviour (circular R_f. 0·37, with butanol, acetic acid and water at 28°) as well as other colour reactions with a synthetic sample of nornobiletin.[6]

The norcompound, on acetylation with acetic anhydride in pyridine, gave the acetate which crystallized from alcohol as colourless needles, m.p. 226–28°.[7]

Fraction B′ (*auranetin*, I). This fraction crystallized from alcohol as colourless needles, m.p. 139–140° (0·5 g). Mixed m.p. with auranetin was undepressed.

Fraction C′ (*hesperidin*). It crystallized from pyridine, m.p. 250–51° and agreed with an authentic sample of hesperidin in all respects.

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[19] K. Josephson, *Arkiv Kemi, Min. Geol.* **36**, 11 (1927).