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CHEMICAL INVESTIGATION OF *CITRUS MITIS* BLANCO—III

ISOLATION OF TWO NEW FLAVANONES

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Abstract—From the peels of *Citrus mitis* Blanco., two new flavanones, Citromitin (5,6,7,8,3',4'-hexamethoxyflavanone) and 5-O-desmethylcitromitin (5-hydroxy-6,7,8,3',4'-pentamethoxyflavanone) were isolated by extraction with petroleum ether.

THE ripe fruit of *Citrus mitis* Blanco.[1,2] is reddish orange in colour and the size of a lemon. The weight of a fruit varies from 25–30 g and the juice and peels form 20–25 per cent and 30–37 per cent respectively of the weight of the fruit.

The similarity of *C. mitis* Blanco. to kamala (*Citrus aurantium*) and the lack of chemical information prompted the investigation. The petroleum ether extract of the peels furnished a bright yellow crystalline solid which could be separated into a neutral compound (A), a phenolic compound (B) and a third crystalline compound (C) identification of which could not be achieved on account of poor yield.

Citromitin ($C_{21}H_{24}O_8$, $\frac{1}{2}H_2O$; m.p. 134–136°) was considered to be a flavanone as it developed a deep red violet colour with sodium borohydride and concentrated hydrochloric acid.[3] Functional group analysis indicated six methoxyls in the molecule. As no hexamethoxyflavanone is known to occur in nature, compound A was considered to be a new flavanone and hence named Citromitin.

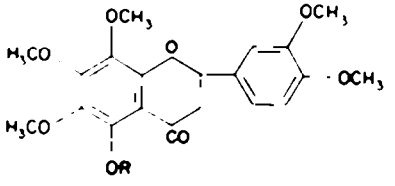
Citromitin could be readily converted into the corresponding chalkone ($C_{21}H_{24}O_8$, 1-H_2O; m.p. 116–117°) which on oxidation with pyridine and iodine or with alkaline hydrogen peroxide furnished veratric acid. During prolonged alkaline hydrolysis of citromitin, the chalkone was formed as the major product (68 per cent) along with a minor quantity of veratric acid (17 per cent). The chalkone character of the hydrolytic product was further established by its reconversion into citromitin by refluxing with 3 per cent alcoholic sulphuric acid. These facts suggest that citromitin is a flavanone with a 3',4'-dimethoxyphenylside nucleus. If out of the six methoxyls two are thus fixed, the remaining four must be present in the benzopyrone structure presumably in 5, 6, 7, 8, positions. Citromitin may, therefore, be represented as 5,6,7,8,3',4'-hexamethoxyflavanone (I, R = CH_3) and the chalkone as 2-hydroxy-3,4,5,6,3',4'-hexamethoxychalkone (II).

A hexamethoxyflavanone of this constitution (I, R = CH_3) and the corresponding chalkone (II) were synthesized earlier by Oliverio and Casinovi[4] who reported melting points (119° and 97–98°) much lower than that of citromitin and its chalkone. For comparison with an authentic specimen the synthesis was repeated and the synthetic 2-hydroxy-3,4,5,6,3',4'-hexamethoxychalkone (II, m.p. 112–114°) was identical with

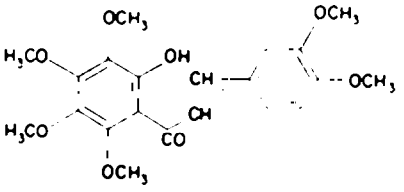
[1] G. P. Sastry and L. R. Row, *J. Sci. Ind. Res.* **19B**, 500 (1960).
[2] G. P. Sastry and L. R. Row, *J. Sci. Ind. Res.* **20B**, 187 (1961).
[3] Robert M. Horowitz, *J. Org. Chem.* **22**, 1733 (1957).
[4] A. Oliverio and C. Casinovi, *Chem. Abstr.* **46**, 978b (1952).

the chalkone from citromitin. It was cyclized as before and the resulting flavanone (I, R = CH_3; m.p. 132–134°) was identical with natural citromitin.

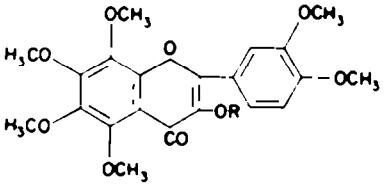
Citromitin is the first completely methylated flavanone to be isolated from the citrus fruits and forms the second known flavanone with 5, 6, 7, 8, substitution, the first being isopedicin[5] (6-hydroxy-5,7,8,-trimethoxyflavanone) occurring in the leaves of *Didymocarpus pedicellata* R.Br. (*Gesneraceae*) along with its chalkone, pedicin.



I



II



III

It may be of interest to mention that the completely methylated chalkone, pedicellin, also occurs in the leaves of the same plant. Closely related flavones, nobiletin,[6] (5,6,7,8,3′,4′-hexamethoxyflavone), 5-O-desmethylnobiletin[7] as well as the lower member, ponkanetin,[8] (5,6,7,8,4′-pentamethoxyflavone), have been isolated from the citrus fruits.

The flavanone character of citromitin was further proved by converting it into the corresponding 3-hydroxyflavone (III, R = H) by the modified Kostanecki's synthesis of Row and Seshadri.[9] The 3-hydroxy-hexamethoxyflavone thus obtained gave rise to a heptamethoxyflavone (III, R = CH_3) m.p. 129–130°, agreeing with that already recorded in literature.[10] Selenium dioxide oxidation[8] of citromitin furnished nobiletin in good yield.

Compound B ($C_{20}H_{22}O_8$, m.p. 146–147°) is also a flavanone as it exhibits reddish pink colour with sodium borohydride and hydrochloric acid.[3] Analysis indicated five methoxyls in the molecule and complete methylation yielded a hexamethoxyflavanone identical with citromitin. From the ferric chloride colouration (greenish brown), compound B may be regarded as 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavanone (I, R = H) and hence named 5-O-desmethylcitromitin. This constitution (I, R H) for compound B was shown to be correct by its synthesis from citromitin. The latter was demethylated with hydriodic acid to give hexahydroxyflavanone which could be remethylated back to citromitin. The formation of a stable hexahydroxyflavanone during demethylation suggests that the stability could have been

[5] K. V. Rao and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **27A**, 375 (1948).
[6] K. F. Tseng, *J. Chem. Soc.* 1003 (1938).
[7] P. S. Sarin and T. R. Seshadri, *Tetrahedron* **8**, 64, (1960).
[8] J. M. Sehgal, T. R. Seshadri and K. L. Vadhera, *Proc. Ind. Acad. Sci.* **42A**, 252 (1955).
[9] L. R. Row and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **21A**, 248 (1945).
[10] V. Venkateswarlu and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **23A**, 354 (1946).

caused by chelation between the 5-hydroxyl and the carbonyl group.[11] Methylation of the hexahydroxyflavanone with excess diazomethane gave rise to a 5-hydroxy-pentamethoxyflavanone (I, R = H) melting at 146–147°, alone or mixed with compound B.

EXPERIMENTAL

The sun-dried peels (4 kg) of *C. mitis* Blanco were powdered in a disintegrator and exhausted successively with pet ether (b.p. 40–60°), ether and methanol in a large soxhlet extractor.

Examination of petroleum ether extract. Upon concentration, the extract deposited a yellow crystalline solid in an hour. After 48 hr, the solid was collected at the pump and shaken with 3% aqueous alcoholic (1:1) sodium hydroxide. The alkaline layer was repeatedly extracted with ether and the total ether extract (800 ml) evaporated to get the neutral compound (A); yield: 8·2 g. The alkaline layer was acidified and extracted with ether. Evaporation of the ether deposited a phenolic compound (B); yield: 1·1 g.

The mother liquor was steam distilled to collect the orange peel oil. The residue was extracted several times with pet ether (10 × 100 ml), the extract washed with conc HCl (5 × 25 ml) and the washings dropped into ice-cold water. The separated solid is designated compound C; yield: 150 mg.

Examination of compound A

Citromitin. Compound A was crystallized thrice from methanol and finally from benzene–pet ether (1:4) as fine lemon yellow needles m.p. at 134–136°. (Found: C, 61·19; H, 5·95; OCH_3, 45·33; $C_{21}H_{24}O_8$–$\frac{1}{2}H_2O$ requires: C, 61·01; H, 6·05; 6-OCH_3, 45·03%). U.V. Spectrum in 95% ethanol[12] has λ_{max} 335, 272 (inflexion) and 250 mμ (log ε, 4·38, 4·23, and 4·32). It gave no colour with alcoholic $FeCl_3$, and a bright reddish violet colour with sodium borohydride and HCl and also with Mg–HCl.

Alkaline hydrolysis of citromitin. A solution of citromitin (2 g) in 8% absolute alcoholic potassium hydroxide (60 ml) was refluxed for an hour on a water bath. Alcohol was removed under red press and HCl added. The resulting bright yellow crystalline solid separated from methanol after two crystallizations as bright orange flakes; m.p. 116–117°; yield: 1·65 g (Found: C, 59·99; H, 5·90; OCH_3, 44·29; $C_{21}H_{24}O_8$–$1H_2O$ requires: C, 59·71; H, 6·16; 6-OCH_3, 44·08%). U.V. Spectrum in 95% ethanol has peaks at λ_{max} 382 and 288 mμ (log ε, 4·30 and 4·09). It developed a brown colour with alcoholic $FeCl_3$ and with sodium borohydride–HCl only a yellow colour.

When the hydrolysis of citromitin was repeated refluxing the liquid for 8 hr, a small quantity of veratric acid (17%) was obtained along with the chalkone (68%).

Conversion of the chalkone into citromitin. A solution of the above chalkone (500 mg) in 3% alcoholic sulphuric acid (50 ml) was refluxed for 30 hr. Removal of the solvent under red press followed by addition of water deposited a pale yellow solid which crystallized from methanol as lemon yellow needles, m.p. 134–136°, undepressed by citromitin. Yield: 450 mg.

Synthesis of 2-hydroxy-3,4,5,6,3′,4′,-hexamethoxychalkone

To a solution of 2-hydroxy-3,4,5,6,-tetramethoxyacetophenone[13] (200 mg) and veratric aldehyde (1·3 g) in ethanol (10 ml) was added aqueous sodium hydroxide (3 g in 3 ml) in small quantities while cooling in ice. The flask was corked airtight and kept at room temp for 2 days. The deep red viscous liquid was worked up as usual. The chalkone crystallized from methanol as orange yellow crystalline plates, m.p. 112–114°, unchanged by the chalkone from citromitin. Yield; 85 mg. (Found: C, 59·85; H, 5·98%).

The chalkone (45 mg) was refluxed with 3% alcoholic sulphuric acid (10 ml) for several hours; the product was a pale yellow crystalline solid, m.p. 130–133° undepressed by admixture with citromitin.

Demethylation of citromitin: 5,6,7,8,3′,4′-*hexahydroxyflavanone*

Hydriodic acid (50 ml *d.* 1·7) was added to a solution of citromitin (1 g) in acetic anhydride (10 ml) and refluxed for 3 hr, cooled and diluted with a solution of sodium bisulphite. The hexahydroxyflavanone was filtered and crystallized from aqueous acetic acid in the form of yellow crystalline solid

[11] N. N. Chari and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **27A**, 223 (1940).
[12] The U.V. measurements reported in this paper were made on Hilgers Uvispek photoelectric colorimeter.
[13] W. Baker, *J. Chem. Soc.* 666 (1941).

which did not melt below 340°; yield 540 mg. (Found: C, 52·05; H, 4·33; $C_{15}H_{12}O_6$–$1\frac{1}{2}H_2O$ requires: C, 51·88; H, 4·32%). It developed greenish brown colour with alcoholic $FeCl_3$, bright red with Mg–HCl and orange red with sodium borohydride and HCl. It changed yellow to greenish brown in 5% sodium bicarbonate, yellow to brownish yellow in 5% sodium hydroxide (10 sec) and red to deep crimson red in 5% sodium carbonate (40 sec).

The hexaacetyl derivative (Ac_2O and Na Ac) crystallized from methanol as colourless needles, m.p. 225–227°. (Found: C, 57·02, H, 4·51; $C_{27}H_{24}O_{14}$ requires: C, 56·63; H, 4·20%).

Methylation of 5,6,7,8,3',4'-*hexahydroxyflavanone*

(a) *Synthesis of* 5-O-*desmethylcitromitin.* An ethereal solution of diazomethane was added in excess to an alcoholic solution of the hexahydroxyflavanone (500 mg). After 36 hr at 0°, the solvent was evaporated and the residue crystallized from methanol as light yellow rhombic plates, m.p. 146–147°, undepressed by compound B, yield: 450 mg. (Found: C, 61·64; H, 5·82; OCH_3, 39·16; $C_{20}H_{22}O_6$ requires: C, 61·51; H, 5·60; 5-OCH_3, 39·74%). It exhibited a brown colour with alcoholic $FeCl_3$.

(b) *Remethylation to citromitin.* The hexahydroxyflavanone (200 mg) was refluxed for 12 hr with anhydrous potassium carbonate (3 g) and dimethyl sulphate (0·9 g) in acetone solution. The methyl ether was separated as usual and crystallized from benzene–pet ether (1:4) as lemon yellow needles, m.p. 134–136° unchanged by citromitin, yield: 150 mg.

Synthesis of 3-*hydroxy*-5,6,7,8,3',4'-*hexamethoxyflavone*

To a gently boiling solution of citromitin (500 mg) in alcohol (50 ml), freshly distilled amyl nitrite (3·5 ml) and conc HCl (*d* 1·19; 35 ml) were added alternately in small quantities with stirring. The solution turned bright yellow at first and finally bright reddish orange. The hot solution was cooled and diluted. It was extracted with ether several times and the ether extract shaken with 3% alkali. On acidification of the alkaline layer an yellow solid was obtained which crystallized from alcohol in the form of prismatic rods, m.p. 141–143°, yield 100 mg. (Found: C, 60·43; H, 5·51; OCH_3, 45·12: $C_{21}H_{22}O_8$ requires: C, 60·29; H, 5·26; 6-OCH_3, 44·49%). It developed brown colour with alcoholic $FeCl_3$.

3,5,6,7,8,3',4'-Heptamethoxyflavone. The above 3-hydroxyflavone (100 mg) was refluxed for 4 hr in acetone (30 ml) with potassium carbonate (1 g) and dimethyl sulphate (0·6 ml). The methyl ether crystallized from alcohol as colourless needles, m.p. 128–129°; yield: 80 mg. (Found: C, 61·19; H, 5·99: $C_{22}H_{24}O_8$ requires C, 61·10; H, 5·56%). No colour was developed with alcoholic $FeCl_3$; but only a pale yellow colour with sodium borohydride and HCl.

Selenium dioxide oxidation of citromitin. Citromitin (340 mg) in isoamyl alcohol (8 ml) was refluxed with selenium dioxide (340 mg) for 12 hr at 140–145°. Metallic selenium was filtered off and the filtrate steam distilled to remove the amyl alcohol. The residue crystallized from methanol as colourless needles, m.p. 134–136°; mixed m.p. with citromitin 82–117°. It did not answer the colour test with sodium borohydride and HCl for flavanone. Tseng[4] records m.p. 134° for nobiletin. (Found: C, 62·42; H, 5·63; $C_{21}H_{22}O_8$ requires: C, 62·69; H, 5·47%).

Examination of compound B

5-O-*Desmethylcitromitin.* Compound B crystallized from methanol as light yellow rhombic plates, m.p. 146–147° undepressed by synthetic 5-O-desmethylcitromitin (see above). (Found: C, 61·82; H, 5·92; OCH_3, 40·05; $C_{20}H_{22}O_8$ requires: C, 61·51; H, 5·60; 5-OCH_3, 39·74%). U.V. Spectrum in 95% ethanol has λ_{max} 344 and 283 and 255 mμ (log ϵ, 4·37, 4·28 and 4·19). It gave dark brown colour with alcoholic $FeCl_3$, bright violet colour with Mg–HCl and reddish pink colour with sodium borohydride and HCl.

Methylation of 5-O-*desmethylcitromitin.* Compound B (200 mg) was methylated in anhydrous acetone (80 ml) with anhydrous potassium carbonate (2 g) and dimethyl sulphate (1 ml). The methyl ether crystallized from methanol; m.p. 134–135° alone or mixed with citromitin.

Examination of compound C

Compound C was crystallized from alcohol as light yellow needles, m.p. 141–143°. (Found: C, 61·93; H, 5·07; $C_{20}H_{20}O_8$ requires: C, 61·80; H, 5·15%). It developed brown colour with alcoholic $FeCl_3$, only a yellow colour with sodium borohydride and HCl and a bright reddish violet colour with Mg–HCl.