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CHEMICAL EXAMINATION OF THE PEEL OF *CITRUS JAMBHIRI* LUSH

ISOLATION OF A NEW FLAVONE

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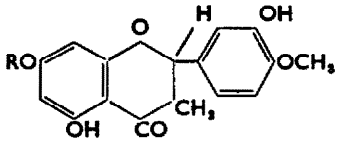
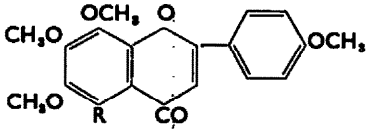
Abstract—The peel of *Citrus jambhiri* lush., a lemon, from Nagaland and from the Assam plains contains both hesperidin and tangeretin. In addition, the plains variety contains neohesperidin. The phenolic compound obtained from the Nagaland variety has been assigned the structure of 5-O-desmethyltangeretin while a phenolic compound isolated from the plains variety has not been investigated.

THE peel of *Citrus limon*[1] contains hesperidin (I)—the only flavonoid isolated from this source. In the peel of *Citrus jambhiri* from Nagaland, besides hesperidin, tangeretin (II) and 5-O-desmethyltangeretin (III) have been isolated.



I, R = 6-O-α-L-rhamnose-D-glucose



II, R = OCH_3
III, R = OH

The chemical constituents from citrus peel are known to differ not only from species to species but also within the same species from locality to locality. Therefore, another variety of the species *Citrus jambhiri* from the plains of Assam, namely Jorhat, also has been examined. Hesperidin and tangeretin are present in both the hill variety (Nagaland) and the variety from the plains. Neohesperidin has been found in the Jorhat variety while a phenolic compound present in this variety is not identical with 5-O-desmethyltangeretin found in the Nagaland variety.

The ripe fruit of *Citrus jambhiri* Lush. (Assamese-Sendurinemutenga) is deep red or vermilion in colour with a smooth surface. It grows abundantly in Nagaland but is found also in the plains adjoining Nagaland.

The crushed dry peel of the fruit (Nagaland variety) was extracted successively with petroleum ether, ether and methanol. The petroleum ether extract yielded β-sitosterol and tangeretin. The ether extract yielded tangeretin and a phenolic compound which proved to be 5-O-desmethyltangeretin by comparison with a synthetic sample. 5-O-Desmethyltangeretin was synthesized by selective demethylation as adopted by Aiyar *et al.*[2] for flavanones. It was also synthesized by oxidizing tangeretin with nitric acid following the procedure of Rao *et al.*[3] wherein the quinone obtained was reduced and partially methylated.

[1] B. P. Chaliha, G. P. Sastry and P. R. Rao, *Indian J. Chem.* 2 (1), 40 (1964).
[2] S. N. Aiyar, I. Dass and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **46A**, 238 (1957).
[3] G. V. K. Rao, K. V. Rao, and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **28A**, 103 (1948).

Earlier studies on the flavonoid constituents of the peels of the citrus fruits revealed the presence of several other 5-hydroxyflavonoid compounds such as, 5-O-desmethylnobiletin, 5-hydroxyauranetin and 5-O-desmethylcitromitin. The first two compounds had been isolated from the peel of *Citrus aurantium*[4] and the third one from the peel of *Citrus mitis* Blanco.[5]

The methanol extract yielded a glycoside which was identified as hesperidin. The R_f value of the glycoside was 0–42 in butanol:acetic acid:water (4:1:5) and agreed closely with that reported by Gaze *et al.*[6] for hesperidin.

A similar examination on the peel of the Jorhat variety yielded four flavonoids—hesperidin, neohesperidin, tangeretin and a phenolic compound the constitution of which could not be established because of very low yield.

EXPERIMENTAL

Nagaland variety of C. jambhiri *Lush.*

Extraction with petroleum ether. The peel (1 kg) was sundried, powdered and extracted with pet. ether (b.p. 40–60°) in a soxhlet extractor for 48 hr. The solvent was concentrated and the resulting reddish orange oil kept for 48 hr in a refrigerator. No solid separated out. Fractionation of this oil by column chromatography using activated alumina as adsorbant and benzene as eluant, yielded compounds A and B. The chromatogram was first washed with pet. ether to remove the essential oil.

Compound A was crystallized twice from MeOH as shining colourless flakes; yield 200 mg, m.p. 136–38°; $[\alpha]_D^{30}$, −35° (1·2, acetone). (Found: C, 83·78; H, 12·40; $C_{29}H_{50}O$ requires: C, 84·04; H, 12·08%). The analysis and optical rotation agree with those of β-sitosterol.

Compound B was crystallized thrice from MeOH as colourless needles m.p. 150–52°, yield 1·0 g. (Found: C, 64·72; H, 5·58; OCH_3, 41·8; $C_{20}H_{20}O_7$ requires: C, 64·5; H, 5·4; 5-OCH_3, 41·6%). The compound gave UV absorption maximum in absolute EtOH at λ 270 mμ (log ε 4·23). It gave no colour with alcoholic $FeCl_3$ and a bright orange red colour with Mg and HCl. Mixed m.p. with an authentic sample of tangeretin was undepressed.

Extraction with ether. The pet. ether exhausted peel was extracted with ether for 48 hr. The solvent was removed and the residual semisolid dissolved in ether and extracted with 5% NaOH aq (4 × 30 ml). The alkali extract was acidified with ice cold HCl aq and extracted with ether. The extract was dried (Na_2SO_4) and after removal of the ether a bright yellow solid (compound C) was obtained.

Compound C was crystallized thrice from MeOH and finally from benzene–pet. ether (1:4) as fine yellow needles m.p. 176–177°, yield 0·5 g. (Found: C, 63·58; H, 5·15; OCH_3, 34·4; $C_{19}H_{18}O_7$ requires: C, 63·68; H, 5·03; 4-OCH_3, 34·63%). It gave UV absorption maxima in absolute EtOH at λ 292 and 330 mμ (log ε 4·40 and 4·35) and in 1% solution of $AlCl_3$ (0·2 ml) at λ 310 and 350 mμ (log ε 4·48 and 4·49). It gave a greenish brown colour with alcoholic $FeCl_3$ and bright orange red colour with Mg and HCl.

Methylation of compound C

(a) *With diazomethane.* Compound C (100 mg) was dissolved in absolute alcohol (35 ml) and excess diazomethane (from 2 g nitrosomethyl urea) in ether was added and the solution kept in a refrigerator overnight. The solution was evaporated and the residue crystallized from alcohol as yellow needles, m.p. 176–178° m.m.p. with compound C was undepressed.

(b) *With dimethyl sulphate.* Compound C (200 mg) was methylated in anhydrous acetone (80 ml) with dimethyl sulphate (0·8 ml) in presence of anhydrous K_2CO_3 (2 g). The mixture was refluxed for 8 hr, the potassium salts filtered off and washed well with acetone. The acetone was evaporated and the methyl ether crystallized from benzene–pet. ether (1:4) as colourless needles m.p. 150–52°, m.m.p. with an authentic sample of tangeretin was undepressed. (Found: C, 64·65; H, 5·55; OCH_3, 41·8; $C_{20}H_{20}O_7$ requires: C, 64·5; H, 5·4; 5-OCH_3, 41·6%).

[4] P. S. Sarin and T. R. Sashadri, *Tetrahedron* **8**, 64 (1960).
[5] G. P. Sastry and L. R. Row, *Tetrahedron* **15**, 111 (1961).
[6] T. B. Gaze, Carl B. Douglass and Simon H. Wender, *Analyt. Chem.* **23**, 1582 (1951).

The fission of the methyl ether (0·1) g was effected by refluxing with alcoholic KOH (20 ml, 8%) for 6 hr. The $NaHCO_3$ aq soluble fraction gave anisic acid, m.p. and m.m.p. 182–184°.

Demethylation of compound C to nortangeretin

Compound C (200 mg) was boiled with HI (10 ml) and acetic anhydride (20 ml). The hydroxyflavone so obtained crystallized from EtOH as yellow plates, m.p. 316–318° (dec.) lit.[7] 318°. (Found: C, 59·71; H, 3·52; $C_{15}H_{10}O_7$ requires: C, 59·6; H, 3·3%). It gave a brown colour with alcoholic $FeCl_3$.

Selective demethylation of tangeretin

Tangeretin (200 mg) was dissolved in anhydrous ether (100 ml) and powdered anhydrous $AlCl_3$ (1 g) added while stirring during the course of 20 min. The mixture was kept at room temperature for 24 hr and then decomposed with ice cold HCl aq. A yellow solid separated which was filtered off washed with water and recrystallized thrice from MeOH as fine yellow needles, m.p. 176–178° undepressed by admixture with compound C.

Oxidation of tangeretin

5,8-*quino*-6,7,4′-*trimethoxy-flavone*. Nitric acid (*d*. 1·2; 12 ml) was added to tangeretin (500 mg) with stirring while cooling in an ice-bath. The mixture was kept 30 min at 15–20° with shaking, filtered and crystallized from alcohol as brick red needles, yield 300 mg, m.p. 228–230°. (Found: C, 63·01; H, 4·23; $C_{18}H_{14}O_7$ requires: C, 63·16; H, 4·12%).

Oxidation of 5-O-*desmethyl tangeretin*

5-O-Desmethyltangeretin (500 mg) was shaken with HNO_3 (*d*, 1·2; 12 ml) for 30 min and the reddish solid which deposited crystallized from alcohol as brick red needles, m.p. 228–230° not depressed by admixture with the quinone from tangeretin, yield 325 mg.

5,8-*Dihydroxy*-6,7,4′-*trimethoxyflavone*. A solution of the above quinoflavone (300 mg) in glacial acetic acid (2 ml) was treated with $NaHSO_3$ (500 ml) warmed for a few min, diluted with water after 5 min and the resulting yellow solid crystallized from alcohol, yield 150 mg, m.p. 245–247° (lit. 250–251°)[7]. (Found: C, 62·1; H, 4·7; $C_{18}H_{16}O_7$ requires: C, 62·8; H, 4·6%).

Methylation of 5,8-*dihydroxy*-6,7,4′-*trimethoxyflavone*

(a) *With diazomethane*. 5,8-Dihydroxy-6,7,4′-trimethoxyflavone (50 mg) was dissolved in absolute alcohol (15 ml) and excess of an ethereal solution of diazomethane (from 2g nitrosomethyl urea) was added and the mixture kept 24 hr at 0°. The solvent was distilled off and the residue crystallized from alcohol as yellow needles, yield 30 mg, m.p. 174–176°, not depressed on admixture with 5-O-Desmethyltangeretin. (Found: C, 63·38; H, 5·26; $C_{19}H_{18}O_7$ requires: C, 63·68; H, 5·03%).

Complete methylation

5-8-Dihydroxy-6,7,4′-trimethoxyflavone (100 mg) was dissolved in dry acetone (20 ml) and anhydrous K_2CO_3 (1 g) and dimethyl sulphate (0·9 g) added. The mixture was refluxed for 24 hr. The methyl ether was worked up in the usual manner and crystallized from MeOH as white needles, yield 75 mg, m.p. 150–151° unchanged on admixture with tangeretin.

Extraction with methanol. The ether exhausted peel was extracted with MeOH for 48 hr. The extract was concentrated to a small volume and kept until a pale brown solid (0·8 g) separated out. This was crystallized twice from glacial acetic acid as white colourless needles, yield 0·75 g, m.p. 252–54° agreeing in all respects with an authentic sample of hesperidin.[1] $[\alpha]_D^{30}$, −87·5° (*c*, 0·752, pyridine); R_f value = 0·42. (Found: C, 53·12; H, 5·28; OCH_3, 5·10; $C_{28}H_{34}O_{15}.H_2O$ requires: C, 53·5; H, 5·7; OCH_3, 4·93%). On acid hydrolysis it gave hesperetin m.p. and m.m.p. 224–225°, D-glucose (R_f = 0·28) and rhamnose (R_f = 0·48).

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[7] V. V. S. Murty, K. V. Rao and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **26A**, 182 (1947).