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SIX NEW FLAVONOIDS FROM *CITRUS*

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Abstract—Valencia orange (*C. sinensis*) and Robinson tangerine [(*C. paradisi* × *C. reticulata*) × (*C. reticulata*)] were examined for flavonoids. Thirteen flavonoids were isolated, six of which are new constituents of citrus peel. These are: 3,5,6,7,3′,4′-hexamethoxyflavone, 3,5,7,8,3′,4′-hexamethoxyflavone, 5-hydroxy-3,7,8,3′,4′-pentamethoxyflavone, 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone, 5,7,8,4′-tetramethoxyflavone and 5,7,8,3′,4′-pentamethoxyflavone. The latter three flavonoids are reported for the first time as natural products. A method is described for readily obtaining small quantities of 5,7,8,4′-tetramethoxy and 5,7,8,3′,4′-pentamethoxyflavones from their 5,6,7-trimethoxy analogs.

INTRODUCTION

THE FLAVONOID fraction of citrus peel has been studied extensively.[1-3] Swift[1] reported five polymethoxyflavones from Hamlin, Parson Brown, Pineapple and Valencia oranges. Three 5-hydroxy-polymethoxyflavones have been found in *Citrus*, two in *C. aurantium*[2] and one in *C. jambhiri*.[4]

During a study on preparation of color extracts from citrus peel,[5] a fraction rich in flavonoids was obtained. TLC showed it to contain many minor components that had not been examined previously. The current study was undertaken to examine this crude flavonoid fraction. The isolation and identification of 13 flavonoids from citrus peel, six of which are new to citrus, is the subject of this report.

RESULTS AND DISCUSSION

Thirteen flavonoids were isolated and identified from Valencia orange and Robinson tangerine peel and peel juice which had been steam stripped of volatiles.[6] These compounds are listed in Table 1. Compounds I, II, VIII, XI, XII and XIII have not been previously reported in citrus. The relative amounts of these compounds present in each of the two citrus peels examined are shown as mg of pure sample isolated by preparative TLC from

* One of the laboratories of the Southeastern Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture.

[1] L. J. SWIFT, *Agric. Food Chem.* **15**, 99 (1967).
[2] R. M. HOROWITZ, in *The Orange* (edited by W. B. SINCLAIR), University of California Printing Department (1961).
[3] P. S. SARIN and T. R. SESHADRI, *Tetrahedron* **8**, 64 (1960).
[4] B. P. CHALIHA, G. P. SASTRI and P. R. ROA, *Tetrahedron* **21**, 1441 (1965).
[5] C. W. WILSON, III, O. W. BISSETT and R. E. BERRY, *J. Food Sci.* **31**, 1033 (1971).
[6] M. K. VELDHUIS, R. E. BERRY, C. J. WAGNER, JR., E. D. LUND and W. L. BRYAN, *J. Food Sci.* **37**, 108 (1972).

extracts (see Experimental). Compounds I, II and IV were isolated from hexane extracts of peel but were not isolated from the benzene extracts of citrus peel or peel juice.

5,7,8,4′-Tetramethoxyflavone (XII) and 5,7,8,3′,4′-pentamethoxyflavone (XIII) had not been previously isolated as natural products. However, the 5-hydroxy analog of compound XIII, 5-hydroxy-7,8,3′,4′-tetramethoxyflavone, had been isolated from Bergamot oil,[7] while compounds XII and XIII had both been previously synthesized.[8,9] A synthetic sample of 5,7,8,4′-tetramethoxyflavone (XII) for comparison with that isolated from citrus peel was prepared from 5-hydroxy-7,4′-dimethoxyflavone by nuclear oxidation[10] to 5,8-dihydroxy-7,4′-dimethoxyflavone followed by methylation of that product with diazomethane. This nuclear oxidation reaction using potassium persulfate in aqueous KOH following the procedure of Seshadri[11] was initially unsuccessful. However, by using fresh potassium persulfate and less KOH[10] the desired product was obtained in *ca.* 10% yield.

Treatment with HI converts a 5,7,8-trimethoxyflavone to a 5,6,7-trihydroxyflavone.[12] Since acid hydrolysis of saponarin[13] (4′,5,7-hydroxy-6-*C*-glucosylflavone-7-*O*-glucoside) gives an equal mixture of isomers, isovitexin (4′,5,7-hydroxy-6-*C*-glucosylflavone) and vitexin (4′,5,7-hydroxy-8-*C*-glucosylflavone), it seemed probable that 5-hydroxy-6,7-dimethoxy derivatives might be treated with acid to afford 5-hydroxy-7,8-dimethoxyflavone derivatives. Treatment of 5,6,7,4′-tetramethoxyflavone (VII) with acetic acid and hydrochloric acid[10] and separation of the reaction mixture by TLC did afford 5-hydroxy-7,8,4′-trimethoxyflavone in 1·4% yield in addition to starting material and the major product, 5-hydroxy-6,7,4′-trimethoxyflavone. When the latter compound was treated with acetic acid and hydrochloric acid, an 8% yield of 5-hydroxy-7,8,4′-trimethoxyflavone was obtained.

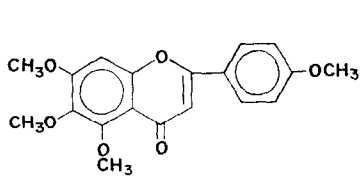
TABLE 1. FLAVONOIDS ISOLATED FROM *Valencia* ORANGE ROBINSON TANGERINE

Methoxyflavones	Found in orange mg pure compound*	Found in tangerine mg pure compound†	Color under UV light
I 50H,3,7,8,3′4′	(2·7)‡		Absorbs
II 50H,3,6,7,8,3′,4′	(3·0)		Absorbs
III 50H,6,7,8,3′,4′		10·3	Absorbs
IV 50H,6,7,8,4′		(4·0)	Absorbs
V 5,6,7,8,4′	5·6	107	Orange
VI 3,5,6,7,8,3′,4′	5·4	10	Grey
VII 5,6,7,4′	5·9		Pink-white
VIII 3,5,6,7,3′,4′	6·0		Blue
IX 5,6,7,8,3′,4′	37·7	286	Grey
X 5,6,7,3′,4′	35·7	53	Blue-white
XI 3,5,7,8,3′,4′	0·9		Red
XII 5,7,8,4′	0·5	53	Red
XIII 5,7,8,3′,4′	1·5	18	Red

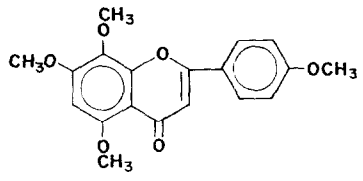
* Isolated from peel juice and is not related to a given weight of peel.
† Isolated from 1·3 kg of tangerine peel.
‡ Parentheses indicate isolation from a hexane extract.

[7] S. FAIRD, *Tetrahedron* **24**, 2121 (1968).
[8] V. D. NAGESWARA SATRI and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **24A**, 243 (1946).
[9] A. OLIVERO, G. B. MORINI BETTOLE and G. BARGELLINI, *Gazz. Chim. Ital.* **78**, 363 (1948).
[10] A. C. JAIN and T. R. SESHADRI, *J. Indian Chem. Soc.* **39**, 515 (1962).
[11] V. V. S. MURTI and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **27A**, 217 (1948).
[12] T. A. GEISSMAN, *The Chemistry of Flavonoid Compounds*, p. 185, Pergamon Press, Oxford (1962).
[13] M. K. SEIKEL and T. A. GEISSMAN, *Arc. Biochem. Biophys.* **71**, 17 (1957).

The same procedure was applied to 5,6,7,3′,4′-pentamethoxyflavone to afford the desired product, 5-hydroxy-7,8,3′,4′-tetramethoxyflavone. The technique of preparative TLC used in this study permitted isolation and identification of some of these minor reaction products not previously observed.[10]



(VII)



(XII)

As shown in Table 1 three flavonoids are found in orange, VII, VIII and X, with the 5,6,7-arrangement and three isomers, XI, XII and XIII, with the 5,7,8-arrangement. The 5,6,7-isomer was found in larger quantity in all three cases in orange. In tangerine, 5,6,7,3′,4′-pentamethoxyflavone (X) (53 mg) predominated over its isomer, 5,7,8,3′,4′-pentamethoxyflavone (XIII) (18 mg), but 53 mg of 5,7,8,4′-tetramethoxyflavone (XII) was found and none of its 5,6,7,4′-tetramethoxy isomer could be detected. A small quantity of a 5-hydroxypentamethoxy flavone was isolated from orange that appeared to be compound III but positive identification was not made.

When the benzene extract of citrus peel juice from Valencia orange was examined by TLC, band I R_f 0·89 (see Table 2) contained a large amount of sterols and two more separations were required to obtain the flavones. Solvent C removed the sterols. Some of the sterols were converted to trimethylsilyl derivatives by Nagy[14] and analysis showed the presence of sitosterol, isofucosterol, stigmasterol, campesterol and cholesterol with 90% of the mixture being sitosterol.

TABLE 2. THICK LAYER CHROMATOGRAPHY OF ORANGE AND TANGERINE EXTRACTS

First TLC total extract solvent A Band No.	R_f 18 cm	Compounds in orange Table 1	Second and third TLC of Bands 1–9 solvents	First TLC total extract Solvent A Band No.	R_f 18 cm	Compounds in tangerine Table 1	Second TLC of Bands 1–8 solvents
1	0·89	I, II	C,D	1	0·89	III, IV	D
2	0·80	V	C	2	0·79	V, VI	B
3	0·75	V, VI	B	3	0·69	V, IX	B
4	0·68	V, VI, VII, VIII	B	4	0·60	V, IX, X	B
5	0·64	VII, IX	B	5	0·52	X, *x*, *y*	A
6	0·57	VII, IX, X	B	6	0·42	X, *x*, *y*, XII	A
7	0·36	XI, XII, XIII	A	7	0·25	XII	A
8	0·30	XI, XII	A	8	0·13	XIII	A
9	0·22	XIII	A				

x, *y* = unknowns.

Total extract spotted and developed and bands collected. Each band was spotted, developed and collected. The compounds were then crystallized. Only Band 1 from orange required a third TLC separation. Chromatography was by multiple development solvent A × 2, other solvents × 4.

EXPERIMENTAL

Preparation of samples. Valencia peel juice centrifuge effluent (9082 l.), a liquid waste from citrus peel oil mills, was used as a source of starting material.[6] The oil and water soluble aromatic fractions were stripped off and the residue concentrated in a pilot plant evaporator. 45 l. of material of approx. 24% solids was obtained and the last 7·5 l. out of the evaporator which was 35·5% solids was collected separately. To 500 g of the 35·5% Brix concentrate was added 150 g NaCl and the mixture was stirred for 10 min. 300 ml of

[14] S. NAGY and H. E. NORDBY, *Lipids* **6**, 826 (1971).

acetone was added and mixing continued for 10 min. The mixture was allowed to stand for 30 min and then exhaustively extracted with 300 ml portions of benzene with acetone added as needed to break emulsions that formed. The extracts were filtered through fluted paper and the filtrate taken to dryness. To the filtrate was added 180 ml isopropanol and 120 ml H_2O. This solution was extracted with three 20-ml portions of hexane to remove carotenoids.[15] The isopropanol water solution was taken to dryness, 100 ml H_2O added, and heated on a steam bath; 150 ml benzene was added and the total mixture was then extracted with benzene (4 × 150 ml). The combined benzene extracts were dried and the solvent removed by evaporation. Yields on duplicates were 1·20 g and 1·10 g. These fractions were transferred to 10 ml volumetric flasks and made up to volume with benzene for TLC. Of the 1·2 g sample, 0·45 ml was spotted on each of six plates, or a total of 297 mg of solids. Compounds V–XIII on Table 1 were isolated with a combined weight of 99 mg or 33% recovery by weight of pure components. Tangerine peel extracts were prepared as follows. To 9·9 kg of ground Robinson tangerine peel was added an equal weight of hexane. This was mixed for 10 min and then run through a juice finisher (with 0·01 cm screen). This hexane extract was used for the preparation of a color extract.[5] To the peel from the finisher was added 19 l. of benzene, this was mixed for 10 min. The peel was put through the finisher and the benzene extract collected. This extract was concentrated on a pilot plant rotary evaporator at 50° and 10 mm Hg. Three successive 500-ml portions of water were added to the mixture and the water distilled at 50° to remove the tangerine oil. The remaining material was taken up in ethanol and placed in 0° storage. A ppt weighing 3·7 g formed in this extract. The material was filtered and the solid was saved. The mother liquor was concentrated by evaporation under vacuum and the residue taken up in 300 ml isopropanol and 200 ml H_2O. This solution was extracted with hexane (3 × 20 ml) to remove the carotenoids. The isopropanol–H_2O solution was then concentrated under vacuum to a heavy syrup. Three 50-ml portions of acetone were added to the syrup and removed under vacuum to remove remaining water. The residue (5·3 g) was taken up in acetone and a portion (1·2 g of solid) was spotted on 12 TLC plates and the compounds III, V, VI, IX, X, XII and XIII isolated. Weight of pure compounds isolated are given in Table 1 and represent 1·3 kg of peel. Other sources of flavonoids came from earlier work on the preparation of color extracts from citrus peel[5] where a flavonoid-containing fraction was obtained from the Robinson tangerine. A similar fraction was obtained from Valencia peel. A portion of the solid (0·8 g) from the hexane extract of Valencia orange peel was separated by TLC. Band 1 (R_f 0·89) contained compounds I and II (2·7 mg and 3·0 mg quantities, respectively). Compounds III and IV were isolated from the tangerine hexane-flavonoids mixture. Compound III was also isolated from the benzene extract of tangerine.

Melting points and spectral analyses. M.ps were determined by placing the sample between glass plates on a Nalge block type apparatus and are uncorrected. IR spectra were taken on KBr pellets. Low resolution MS were obtained on a Bendix Model 3012 Time-of-Flight mass spectrometer at 70 eV ionization potential. High resolution mass spectra were determined with an E.E.I Picker, ultrahigh resolution mass spectrometer at Florida State University. UV spectra were recorded in 95% EtOH.

Preparative TLC procedures. TLC was effected with 20 × 20 cm glass plates coated with 10–40 μ Silica Gel H.F.-254, prepared by using 60 g of adsorbent and 158 ml H_2O. All plate coatings were 1 mm thick. Solvents were (A) benzene–acetone (3:1), (B) hexane–acetone–*n*-BuOH (8:1:1), (C) hexane–acetone–*n*-BuOH (17:2:1) (D) benzene–acetone (49:1) and (E) benzene–acetone (9:1). R_f values were calculated on the basis of the front traveling 18 cm. Multiple development of plates were required in most separations and are indicated in Table 2 as number of passes of solvent. The bands were scraped and eluted as follows: A glass column (300 mm in length and 22 mm i.d.) tapered to 4 mm was plugged with glass wool and this, in turn, covered with a 10 mm layer of sea sand. The scraped bands of adsorbent were placed on the top of the sea sand and eluted with solvent A.

Separation of the various extracts was carried out as follows. The total extract of orange or tangerine was spotted on a thick layer plate and the plate developed in solvent A (2 passes). In the orange extract there were nine major bands. These bands were collected, the components eluted and then spotted on another plate. The bands were then run in the appropriate solvent for further separation. These bands were collected and eluted. Like compounds from the various separations were combined and crystallized. Only Band 1 required a third TLC purification step. The second step for purification of Band 1 was required to remove sterols from the flavonoids. The R_f values are not absolute as they vary with concentration. Thus, the compounds are listed in Table 2 according to their relative R_f in the solvents used with Compound I having the highest R_f and XIII the lowest.

Synthesis of reference compounds. See Table 1. Compounds, V, VI, VII, IX and X were identified by comparing their UV, IR, m.p. and MS to known compounds which were furnished by Swift.[1] The remaining compounds were identified by similar procedures applied to authentic samples or converted to a derivative and compared to knowns.

5-Hydroxy,3,7,8,3′,4′-pentamethoxyflavone (I). Compound I was isolated from orange: UV 360, 274, 257 nm; *m/e* 388, 373 (m-15), m.p. 162–163° (lit. 156–157°).[16] Compound I, 2·5 mg, was dissolved in 20 ml

[15] P. BERNATH and H. E. SWISHER, *Food Tech.* **23**, 107 (1969).
[16] R. M. HOROWITZ, *J. Am. Chem. Soc.* **79**, 6561 (1957).

EtOH plus 40 ml Et_2O and this solution added to an ether solution CH_2N_2.[17] The reaction mixture was left in the hood overnight. One drop HOAc and 20 ml EtOH were added. The solution was warmed on a steam bath and filtered, solvent evaporated and the mixture spotted on a TLC plate: solvent A, two passes of the solvent; product R_f 0·34, starting material R_f 0·81. Product collected and crystallized from EtOH to afford 0·7 mg, indentical in all respects to authentic 3,5,7,8,3′,4′-hexamethoxyflavone obtained from Horowitz (U.S. Fruit and Vegetable Chemistry Laboratory, Pasadena, Calif.).

5-*Hydroxy*-3,6,7,8,3′,4′-*hexamethoxyflavone* (II). Compound II was isolated from orange: UV 345, 279, 258 nm; *m/e* 418, 403 (m-15), m.p. 110–112°, (lit. 110–111° and UV 345, 280, 258).[18] To 163 mg of 3,5,6,7,8,3′, 4′-heptamethoxyflavone was added 10 ml conc. HCl and 10 ml conc. HOAc.[10] The reaction mixture was heated on a steam bath for 2 hr, poured into 75 ml of ice water, extracted twice with 100 ml portions of 50% benzene–ether, the extract dried and the solvent evaporated. The concentrated extract was spotted on two TLC plates. Solvent A, one pass of the solvent. Band 1 R_f 0·66 and Band 2 R_f 0·50, were collected and crystallized from EtOH to yield 67·2 mg of starting material from Band 2 and 28·1 mg of 2 or 17·2% yield from Band 1. This product had: UV 345, 279, 258 nm, ϵ 15 700, 14 900, 14 000, *m/e* 418, 403 (m-15) and m.p. 112–113·5°.

5-*Hydroxy*-6,7,8,3′,4′-*pentamethoxyflavone* (III). Compound III was isolated from tangerine: UV 340, 283, 253, nm, *m/e* 388, 373 (m-15), m.p. 147–148° (lit. m.p. 144–146°).[3] Synthesis of compound III: to 100 mg of 5,6,7,8,3′4′-hexamethoxyflavone were added 10 ml each of conc. HOAc and 10 ml conc. HCl. Treated as synthesis of compound II. Spotted on two TLC plates, solvent A one pass of solvent. Band 1 R_f 0·64 and Band 2 R_f 0·44 were collected and crystallized from EtOH. Band 1 gave 22·2 mg of compound III or 22·2% yield and was identical to III. Band 2 was starting material.

5-*Hydroxy*-6,7,8,4′-*tetramethoxyflavone* (IV). Compound IV was isolated from tangerine: UV 328, 293, 285sh nm, ϵ 23 000, 20 400, 19 900, *m/e* 358, 343 (m-15), and m.p. 177° (lit. m.p. 176–177°)[4] isolated from a *C. jambhiri*). Reported UV 328, 293 nm, ϵ = 21 400, 24 500.[19] To 35 ml of anhydrous ether were slowly added 4 g anhydrous $AlCl_3$, and then 200 mg of 5,6,7,8,4′-pentamethoxyflavone.[20] This mixture was refluxed for 24 hr, then water was very cautiously added and the ether evaporated. The ppt which formed was filtered, washed (H_2O), transferred to a 50 ml flask and 10 ml HOAc and 2 ml HCl were added. The solution was warmed on a steam bath for 5 min, then poured into 75 ml of ice water and a ppt formed immediately. This was filtered and the ppt washed with water and recrystallized from MeOH three times. It was identical to compound IV.

3,5,6,7,3′,4′-*Hexamethoxyflavone* (VIII). Compound VIII was isolated from orange: UV 332, 260sh, 242 nm, *m/e* 402, 387 (m-15), m.p. 140–141°. An authentic sample of 7-hydroxy-3,5,6,3′,4′-pentamethoxyflavone (25 mg) was obtained from Herz[21] (Chem. Dept., Florida State University, Tallahassee, Fla.). This was methylated by the same procedure as compound I to yield 14 mg of compound VIII which was crystallized from EtOH (the product was identical to compound VIII).

3,5,7,8,3′,4′-*Hexamethoxyflavone* (XI). Compound XI was isolated from orange: UV 350, 271, 252 nm, *m/e* 402, 387 (m-15). When crystallized from an excess of EtOH it had an m.p. 171–172°, in agreement with reported data.[22] If the compound was crystallized from a minimum amount of EtOH it had a double m.p.[16] An authentic sample was obtained from R. M. Horowitz (U.S. Fruit and Vegetable Chemistry Laboratory, Pasadena, Calif.) and found identical to XI by UV, IR, MS, R_f and color under UV light.

5,7,8,4′-*Tetramethoxyflavone* (XII). Compound XII was isolated from orange and tangerine: UV 330, 310, 270, 220 nm. ϵ 16 300, 16 700, 20 500, 23 400, *m/e* 342, 327 (m-15), m.p. 209–210° (lit. m.p. 209–210°).[8]

5-*Hydroxy*-7,4′-*dimethoxyflavone* (XIV). To 5 g of 5,7,4′-trihydroxyflavone (apigenin) in 1 l. of anhydrous tetrahydrofuran was added 60 g freshly ignited K_2CO_3 then 3·4 ml Me_2SO_4. Refluxed for 7 hr, filtered and removed the solvent. A solid remained and was taken up in EtOH and crystallized repeatedly. During each recrystallization a small amount of EtOH insoluble material was discarded, 0·85 g of material was collected; m.p. 165–167°. A portion of this material was separated on 4 TLC plates, using solvent E with one pass of solvent (R_f 0·53). A total of 188 mg of 5-hydroxy-7,4′-dimethoxyflavone was collected: UV 330, 268 nm, *m/e* 298, the IR did not show a hydroxyl absorption, m.p. 175–176° (lit. m.p. 174–174·5).[12]

5,8-*Dihydroxy*-7,4′-*dimethoxyflavone* (XV).[10] To 128 mg of 5-hydroxy-7,4′-dimethoxyflavone was added 10 ml pyridine and 10 ml aq. KOH (90 mg/10 ml). This solution was cooled to 10° and then aq. $K_2S_2O_8$ (225 mg/10 ml) was added dropwise over 1-hr period. The reaction mixture was kept at 0° overnight and extracted three times with 30-ml portions of Et_2O to remove pyridine and unreacted starting material. This resulted in recovery of 90 mg of XIV from the Et_2O extract. Conc. HCl was added to the reaction mixture to pH 3·5. It was extracted with ether (3 × 10 ml) to remove side reaction products and starting material, then

[17] T. J. deBoer and H. J. Backer, *Recl. Trav. Chem.* **73**, 232 (1954).
[18] B. Gentili and R. M. Horowitz, *Tetrahedron* **20**, 2313 (1964).
[19] L. Farkas, M. Nogradi, V. Swdarsanam and W. Herz, *J. Org. Chem.* **31**, 3228 (1966).
[20] W. Baker, N. C. Brown and J. A. Scott, *J. Chem. Soc.* 1922 (1939).
[21] W. Herz, *J. Org. Chem.* **26**, 3014 (1961).
[22] T. A. Geissman and C. Steelink, *J. Org. Chem.* **22**, 946 (1957).
[23] N. Morita, *Chem. Pharm. Bulletin* **8**, 59 (1960).

10 ml conc. HCl was added and placed on steam bath. A flocculent ppt began to form at once. The solution was left on a steam bath for 1 hr. The solution was filtered and the product taken up in a minimum amount of hot EtOH. On cooling a ppt formed at once. Collected 6·8 mg product XV, UV 308, 283, 224 nm, ϵ 21 400, 18 800, 17 900, *m/e* 314, a high resolution *m/e* 314·0776 (calc. for $C_{17}H_{14}O_6$ 314·0789), m.p. 269–270°. Compound XV (6 mg) dissolved in 50 ml EtOH and 50 ml Et_2O was slowly added to a CH_2N_2 solution. This was treated as in compound I. The reaction mixture was spotted on TLC plates, solvent A one pass R_f 0·11. The compound was collected and crystallized from EtOH; 3·7 mg the IR and UV identical to compound XII, *m/e* 342, 327 (m-15), and m.p. 212–213°.

5-*Hydroxy*-7,8,4′-*trimethoxyflavone* (XVI) *and* 5-*hydroxy*-6,7,4′-*trimethoxyflavone* (XVII). To 1 g of 5,6,7,4′-tetramethoxyflavone (VII) was added 40 ml conc. HOAc and 10 ml conc. HCl. This was worked up as was compound II. A ppt formed when the solution was poured into ice water. This ppt was collected and the mother liquor extracted as above. Two solid fractions were obtained. Both were impure and were spotted on six TLC plates; solvent A one pass of solvent. Three major bands 1, 2 and 3 R_f 0·78, 0·64 and 0·39 separated. The bands were collected and crystallized from ethanol. Band 1 gave 14·1 mg, Band 2, 183 mg and Band 3 gave 369 mg. Band 1 was 5-hydroxy-7,8,4′-trimethoxyflavone (XVI) UV 322, 302, 274, 22 nm, ϵ 21 500, 19 700, 23 600, 25 600, *m/e* 328, 313 (m-15), and m.p. 222–224°. Methylation and TLC separation (as in synthesis of XV) of 10 mg of 16 gave 4 mg of compound XII. Band 2 was 5-hydroxy-6,7,4′-trimethoxyflavone (XVII); UV 331, 278, 226 nm, ϵ 24 000, 17 700, 31 200, *m/e* 328, 313 (m-15) and m.p. 189–190°. (lit. m.p. 189–190 p. 421).[12] Band 3 was starting material. To 122 mg of XVII was added 10 ml conc. HOAc and 10 ml conc. HCl. This was worked up as above. Band 1 gave 10 mg or 7·8% of XVI and Band 2 gave 71·3 mg or 56% recovery of starting material.

5,7,8,3′,4′-*Pentamethoxyflavone* (XIII). Compound XIII was isolated from orange and tangerine UV 338, 270, 248, 223sh nm, ϵ 20 400, 20 000, 19 200 and 24 700, *m/e* 372, 357 (m-15), and m.p. 197–198° (lit. m.p. 198–199°[9] and 193–194°).[7]

5-*Hydroxy*-6,7,3′4′-*tetramethoxyflavone* (XVIII) *and* 5-*hydroxy*-7,8,3′,4′-*tetramethoxyflavone* (XIX). To 1 g of 5,6,7,3′,4′-pentamethoxyflavone (X) added 40 ml conc. HOAc and 10 ml conc. HCl and heated on a steam bath for 2 hr. This was worked up as in the synthesis of compound II. The extract was spotted on six TLC plates, solvent A, one pass of solvent. Three major bands, Band 1 R_f 0·67, Band 2, 0·56 and Band 3, 0·28. These fractions were collected and crystallized from EtOH. Band 1 yielded 2·0 mg, Band 2, 131 mg and Band 3, starting material 565 mg or 56·5% recovery. Band 1 was 5-hydroxy-7,8,3′4′-tetramethoxyflavone (XIX): UV 340, 290sh, 274, 254, 220sh nm, ϵ 15 100, 9400, 16 300, 14 600, 20 200, *m/e* 358, 343 (m-15) m.p. 207–208° (lit. 210–212°).[7] High resolution *m/e* 358·1040 (calc. for $C_{19}H_{18}O_7$, 358·*1051*. Band 2 was 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (XVIII): UV 341, 276, 242 nm, *m/e* 358, 343 (m-15) m.p. 192–194° (lit. m.p. 189–190°).[23]

5-*Hydroxy*-7,8,3′,4′-*tetramethoxyflavone* (XIX). To 73 mg of 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (XVIII) added 10 ml HOAc and 10 ml conc. HCl. Heated for 2 hr on a steam bath. Poured into 75 ml ice water, no ppt formed. Extracted with three 100-ml portions of 50% benzene–ether. The solvent was removed and the remaining material spotted on two TLC plates, solvent A, two passes of solvent, separation seen on plate only with short wave UV. Band 1 gave 1·8 mg of XIX, a 2·4% yield. Band 2 was concentrated to dryness and treated as above. Collected 3·4 mg of Band 1 and 27·8 mg of starting material, 38% recovery. 48·6 mg of 18 was treated as above except, 20 ml of conc. HCl and no HOAc was used. Collected 1·7 mg of XIX, a 3·5% yield and recovered 20·7 mg of 18 or 43%. 4 mg of XIX was treated with CH_2N_2 and then run on TLC. Solvent A one pass R_f 0·6 and collected the product 2·5 mg of 5,7,8,3′,4′-pentamethoxyflavone (XIII).

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