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PUTRAFLAVONE, A NEW BIFLAVONOID FROM *PUTRANJIVA ROXBURGHII*

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Abstract—A new biflavonoid, named putraflavone (I), was isolated from the acidic fraction of the alcoholic extract of the leaf of *P. roxburghii* Wall. and characterized as 7,4″′-dimethyl amentoflavone with the help of UV, NMR and mass spectrometry.

INTRODUCTION

RECENTLY a number of new triterpenoids belonging to friedelane series have been reported[1–6] from the trunk bark and leaf of *Putranjiva roxburghii*. The systematic chemical examination of the air-dried leaf extract has since yielded a new biflavonoid named putraflavone, m.p. 280–282° on chromatography of the acidic fraction of the extractive over silica gel (eluent, chloroform and chloroform–methanol) besides the keto triterpene acid, roxburghonic acid,[6] $C_{30}H_{48}O_3$, reported earlier.

The properties indicated putraflavone to be a biflavonoid, $C_{32}H_{22}O_{10}$ (M^+ 566) (I) built up of partially methylated units of apigenin. The pale yellow compound gave olive green colour with ferric chloride and orange red with Mg–HCl. In UV light it showed brown colour characteristic of a flavone having possibly no free hydroxyl at C-3.[7] The spectrum of putraflavone showed broad signal for hydroxyl group at 3400 cm^{-1} and the carbonyl band at 1660 cm^{-1}. The UV spectra of putraflavone and its derivatives showed maxima at 218, 270–272 and 336 nm identical to that of apigenin. The corresponding

TABLE 1. UV SPECTRA OF PUTRAFLAVONE AND ITS DERIVATIVES

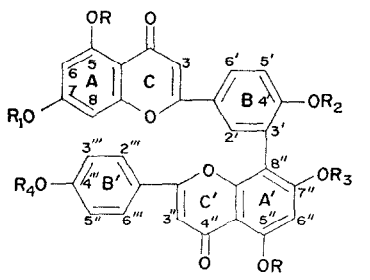
Solvent	Compounds (λ_{max} in nm) I	VII	VIII
EtOH	218, 270, 338	218, 272, 336	215, 270, 336
EtOH–$AlCl_3$*	215, 281, 300, 344, 383	215, 272, 338	215, 282, 299, 340, 370
AcONa	215, 232, 270, 292	218, 270, 338	213, 271, 336
EtONa	218, 230, 276, 295, 376	218, 272, 338	—

* Addition of HCl to the above complex did not show any shift and thus indicated the presence of only 5-OH groups and absence of any 3-OH grouping in the compound.

[1] P. SEN GUPTA, A. K. CHAKRABORTY, A. M. DUFFIELD, L. J. DURHAM and C. DJERASSI, *Tetrahedron* **24**, 1205 (1968).
[2] H. S. GARG and C. R. MITRA, *Phytochem.* **7**, 2053 (1968).
[3] G. R. CHOPRA, A. C. JAIN and T. R. SESHADRI, *Current Sci.* **37**, 301 (1968).
[4] H. S. GARG and C. R. MITRA, *Tetrahedron Letters* 231 (1969).
[5] P. SEN GUPTA and J. MUKHERJEE, *Tetrahedron* **24**, 6259 (1968).
[6] H. S. GARG and C. R. MITRA, *Phytochem.* **10**, 865 (1971).
[7] J. B. HARBORNE, *J. Chromatog.* **2**, 581 (1959).

shifts[8] with $AlCl_3$, $AlCl_3$–HCl, sodium acetate and sodium ethoxide (Table 1) showed the presence of 5, 7 and 4′ free hydroxyl groups in the molecule. This suggested putraflavone to be built up of two apigenin units—the common feature of biflavonoids so far reported[9]—with different linkages.

Putraflavone showed the presence of two methoxy protons at δ3·45 ppm in its NMR spectrum in pyridine which indicated that two of the six hydroxyls of the two units of apigenin were methylated. The NMR spectrum in DMSO showed the presence of twelve protons in the region δ6–8 ppm and the presence of two protons of chelated hydroxyl groups at δ13·15 and 12·9 ppm. Batterham *et al.*[10] have studied the NMR spectra of flavonoids including biflavonoids in detail and have assigned the various protons and noted their chemical shifts due to the presence of different substituent groups. Of the various biflavo-



I R = R₂ = R₃ = H; R₁ = R₄ = CH₃
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V R = R₃ = R₄ = H; R₁ = R₂ = CH₃
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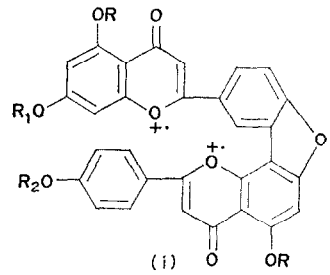
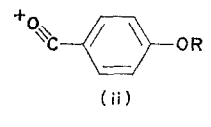
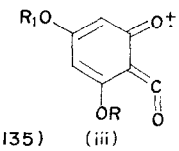


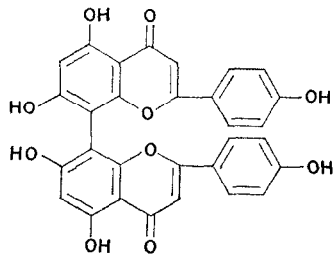
(i)
R = H; R₁ = R₂ = CH₃ (*m/e* 548)
R = R₁ = R₂ = CH₃ (*m/e* 576)



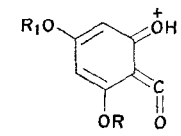
(ii)
R = CH₃ (*m/e* 135)



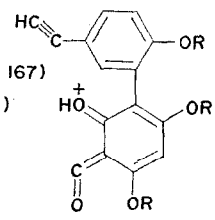
(iii)
R = H; R₁ = CH₃ (*m/e* 166)
R = R₁ = CH₃ (*m/e* 180)



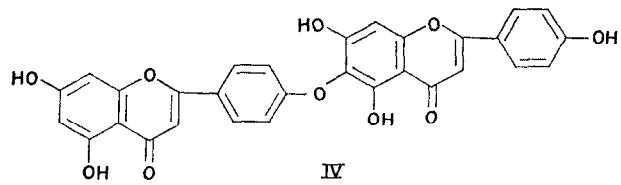
III



(iv)
R = H; R₁ = CH₃ (*m/e* 167)
R = R₁ = CH₃ (*m/e* 181)



(v)
R = CH₃ (*m/e* 311)



IV

[8] L. JURD, *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), pp. 107–130, Pergamon Press, Oxford (1962).
[9] W. BAKER, A. C. M. FINCH, W. D. OLLIS and K. W. ROBINSON, *J. Chem. Soc.* 1477 (1963).
[10] T. J. BATTERHAM and R. J. HIGHET, *Austral. J. Chem.* **17**, 428 (1964).

noids of the bisapigenin reported earlier, the latter belong to three types: the biphenyl types represented by amentoflavone (II)[9] and cupressuflavone (III)[9] and the biphenyl ether pattern represented by hinokiflavone (IV).[9] The general pattern of the NMR spectra of putraflavone was comparable to two known dimethyl derivatives of amentoflavone, namely ginkgetin (V)[10] and isoginkgetin (VI)[10] and is given in Table 2.

In the case of putraflavone, only a total of three protons were detected on two phloroglucinol units as in methyl amentoflavones V and VI, showing that one is used in linking the two units. The proton at δ6·2 (1H, d, $J = 2$ cps) is assigned to C-6 proton of the ring-A showing meta coupling with the free proton at C-8, while the two protons signal at δ6·4 ppm is assigned to C-8, and C-6″ or C-8″ of the ring-A′. The other signals at δ6·7 ppm (4H) may be assigned to two 3‴, 5‴ protons on the phenyl ring-B′ and the third to 5′ of the ring-B and the fourth as C-3 olefinic proton of ring-C. The presence of the other C-3″ olefinic proton of ring-C′ is noted at δ7·2 ppm. The signal at δ7·9 ppm (2H, d, $J = 2$ cps) is assigned to 2′, 6′ protons of the ring-B showing meta coupling while 2‴, 6‴ protons of ring-B′ showed an upfield shift to δ7·5 and 7·6 ppm. These shifts may be attributed to the ring-B′ being methylated and the ring-B being used in the linking of the two units of apigenin. Thus the NMR spectrum of putraflavone is in agreement with the linking of the two units of apigenin through 3′ and 8″ (ring-A′) identical to that of amentoflavone.

The structure was further supported by complete methylation of putraflavone, when it yielded (VII), $C_{36}H_{28}O_{10}$, m.p. 226–228° identical to hexa-methyl amentoflavone, m.p. 227–228°, as evidenced from its NMR (Table 2) and mass spectra (Table 4). Therefore, putraflavone is a dimethyl amentoflavone but different from ginkgetin and isoginkgetin in placement of the methoxyl groups. The comparative melting points of these compounds and their derivatives are given in Table 3. Further, putraflavone, on methylation with diazomethane in presence of methanol yielded 5,5″ dihydroxy-methyl amentoflavone (VIII), $C_{34}H_{24}O_{10}$ (M^+ 594) m.p. 278–280°, showing the presence of two chelated hydroxyl protons at δ13·15 and 12·8 ppm assigned to C-5 hydroxyls[10] of the two units. This indicated the presence of two methoxyl groups in the parent compound at either of the pair of 7 and 4′

TABLE 2. NMR SPECTRA OF PUTRAFLAVONE, GINKGETIN AND ISOGINKGETIN (δPPM)

Proton	Ginkgetin[9a] (DMSO)	Isoginkgetin[9a] (DMSO)	Putraflavone (DMSO)	Methyl putraflavone* ($CDCl_3$)
Ring-A 6	6·37	6·23	6·20	6·45
8	6·80	6·51	6·40	6·60
5-OH	12·96	12·98	12·85	—
Ring-B 2′	8·12	8·09	7·90	8·18
5′	7·38	7·38	6·7	6·92d ($J = 9$ cps)
6′	8·19	8·16	7·9	7·96
Ring-C 3	6·82	6·92	6·7	6·8
Ring-A′ 6″	6·44	6·46	6·4	6·45
5″-OH	13·12	13·13	13·03	—
Ring-B′				
2‴, 6‴	7·51	7·61	7·5d (J = 10 cps)	7·5d ($J = 9$ cps)
3‴, 5‴	6·74	6·97	6·7	6·7, 6·88
Ring-C′ 3″	7·0	6·88	7·2	7·35

* Six methoxyls protons at δ 3·8 (6H), 3·9, 4·0, 4·05 and 4·18 ppm.

[9a] V. V. S. MURTI, P. V. RAMAN and T. R. SESHADRI, *Tetrahedron* **23**, 397 (1967).

TABLE 3

	Amentoflavone	Putraflavone	Ginkgetin	Isoginkgetin
Parent compound	>330°	280–282°	350° (dec)	210° resolidifying
Methyl ether	227–228°	226–228°	—	at 245–247°
5,5″ dihydroxy-methyl ether	281–282°	278–280°	—	finally melting at 353° (dec)

positions as the shifts in UV spectra supported the presence of at least one each of the 5, 7 and 4′ pair as free hydroxyl groups in the two units.

This limits the placement of the two methoxyl groups in putraflavone at positions 7,4′ or 7″,4‴ or 7″,4′ or 7,4‴ in I. Putraflavone being different from ginkgetin, the presence of the methoxyls at 7,4′ positions in the first unit as in the latter was ruled out and similarly the possibility of the 7″,4‴ in the second unit was ruled out on the basis of mass spectral studies with the parent compound as well as its methyl derivative.

Since chemical degradations in these cases do not offer much information, the mass fragmentation of the biflavonoids,[11] cracking pattern of the apigenin and its methyl derivatives being known, was studied in detail, which not only helps in identifying the linkage but also the placement of the functional groups in the two halves of the molecule.

The mass spectra of methyl putraflavone and putraflavone showed the presence of the molecular ion peaks at *m/e* 622 and *m/e* 566, and M^{2+} or $M/2^{+}$ peaks at *m/e* 311 and *m/e* 283, respectively. This suggested the parent compound to be built up of symmetrical units that is each unit having a methoxyl group. The other relevant peaks in methyl putraflavone were identical to that of methyl amentoflavone (Table 4) corresponding to fragments (i) to (v). This fully confirmed the linkage to be 3′ to 8″ in putraflavone.

The RDA cleavage of the molecule in the mass spectra had been of much use in case of biflavonoids which may lead to the major fragments (ii) to (v) as is in case of methyl amentoflavone.[11] The corresponding peaks for these fragments are helpful in elucidating the placement of the functional groups at different positions. The significant mass ion peaks of putraflavone, methyl putraflavone and those of methyl amentoflavone (*loc. cit*) are summarized in Table 4.

In the parent compound as well as its methyl derivative there appeared a prominent peak at *m/e* 135 constituting fragment (ii), which supports the placement of one of the methoxyl groups at 4‴ (ring-B′) in putraflavone. Similarly the other fragment of the RDA cleavage gave rise to peaks at *m/e* 166, 167 in case of putraflavone and at *m/e* 180, 181 in

TABLE 4. MASS SPECTRA (*m/e*)

Putraflavone	Methyl putraflavone	Methyl amentoflavone
566 (M^+)	622 (M^+)	622 (M^+)
548 (M^+-H_2O) (i)	576 (i)	576 (i)
283 (M^{2+}, $M/2^+$)	311 (M^{2+}, $M/2^+$) (v)	311 (M^{2+}, $M/2^+$) (v)
167 (iv)	181 (iv)	181 (iv)
166 (iii)	180 (iii)	180 (iii)
135 (ii)	135 (ii)	135 (ii)

[11] S. NATRAJAN, V. V. S. MURTI and T. R. SESHADRI, *Ind. J. Chem.* **7**, 751 (1969).

case of methyl putraflavone constituting the fragments (iii) and (iv) arising from the free phloroglucinol ring; indicating that position 7 (ring-A) carried a methoxyl group in the parent compound. Thus the two methoxyls could best be placed at 7 and 4‴ positions giving rise to the structure of putraflavone as 7,4‴-dimethyl amentoflavone (I).

This was further supported by the loss of water molecule from molecular ion peak in putraflavone giving rise to a peak at *m/e* 548 corresponding to fragment (i) as in methyl amentoflavone and methyl putraflavone, indicating the absence of the methoxyl groups at 4′ and 7″ positions in putraflavone.

EXPERIMENTAL

Melting points were taken in open capillaries and are uncorrected; UV measurements were recorded on Hilger Uvispek Model 202 in EtOH solution. The NMR spectra were recorded on a Varian A-60 instrument using TMS as internal reference in solvents as mentioned. Mass spectra were recorded by direct inlet system as well as indirect inlet system.

Isolation of putraflavone. The air-dried leaf (5 kg) of *Putranjiva roxburghii* Wall. collected locally was percolated with EtOH (80–85%; 5 l. × 4) and the concentrate (syrupy mass, 1 l.) was separated into Et_2O-soluble and Et_2O-insoluble (aqueous) fractions. The Et_2O-soluble fraction (4 l.) was washed with aq. NaOH (0·5%) to separate it into acidic and neutral fractions. The acidic fraction after usual working yielded the microcrystalline crude flavonoid (1·5 g) which was chromatographed over silica gel column (1:200 w/w) eluting with $CHCl_3$ and $CHCl_3$–MeOH and finally with MeOH when the different compounds were obtained.

The $CHCl_3$ eluted fraction yielded the triterpene roxburghonic acid (350 mg) m.p. > 340°. The elution with $CHCl_3$–MeOH (2:1) afforded the crystalline pale yellow flavonoid, putraflavone (550 mg) m.p. 280–282°, $C_{32}H_{22}O_{10}$(M^+ 566). (Found C, 67·69; H, 3·54; $C_{32}H_{22}O_{10}$ requires C, 67·84; H, 3·89%.)

Methylation of putraflavone with dimethyl sulphate. Putraflavone (150 mg) in dry acetone (40 ml) over K_2CO_3 (10 g) was refluxed at 100° with Me_2SO_4 until methylation complete. Processed in usual manner to yield hexamethyl amentoflavone (methyl putraflavone) m.p. 226–228° (reported m.p. 227–228°) $C_{36}H_{30}O_{10}$ (M^+ 622). (Found C, 69·68; H, 5·02; $C_{36}H_{30}O_{10}$ requires C, 69·45; H, 4·82%.)

Methylation of putraflavone with diazomethane. Putraflavone (120 mg) in MeOH (10 ml) was treated with excess CH_2N_2 at 5°, left overnight at room temp. and processed to yield 5,5″-dihydroxy-methyl amentoflavone, m.p. 278–280° (reported m.p. 281–282°), NMR (pyridine) δ 3·5 (4 × OCH_3) ppm. (Found C, 69·02; H, 4·25; $C_{34}H_{26}O_{10}$ requires C, 68·68; H, 4·39%).

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