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THE CONSTITUTION OF FLAVONOIDS FROM <u>GARCINIA SPICATA</u> HOOK. f.

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The yellow pigment isolated from the bark of Garcinia spicata Hook. f. (Guttiferae)-- Japanese name: fukugi-- was first chemically investigated by Perkin and Phipps[1] who isolated a flavone derivative, m.p. 288-289°, which was named fukugetin. Later Murakami and Irie[2] proposed the structure Ia or Ib for fukugetin, while Kubota and Arai[3] presented a different structure Ic. However, in our re-examination of the constituents of fukugi, we have obtained a dl-form and a d-form of a biflavonoid compound, namely compound (I), m.p. 298-299° (decomp.), $[\alpha]_D^{29}$ 0° (MeOH), and compound (II), m.p. 244-245° (decomp.), $[\alpha]_D^{29}$ + 170° (MeOH), whose structures were clarified according to the following experimental data. Adopting the name used by the previous workers, we call these compounds (±)-fukugetin and (+)-fukugetin, respectively. Besides (±)-3'-O-methyl fukugetin (III), m.p. 290-291° (decomp.), $[\alpha]_D^{25}$ 0°(MeOH), was also isolated.

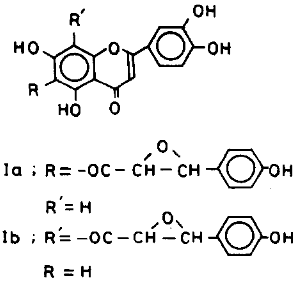
Compounds I, II and III were obtained by repeated polyamide chromatography (methanol as solvent) of the methanol extracts of the bark and fractional recrystallizations,[a] while silica gel chromatography of the ether soluble fraction of the acetone extract of the bark gave a mixture of II and III in a ratio of 20:3.[b] (±)-Fukugetin (I), $C_{30}H_{20}O_{11}$ has UV maxima (MeOH) at 224 (log ε 4.57, shoulder), 275 (4.33), 288 (4.35) and 345 mμ (4.13), and IR bands (nujol mull) at 3250 (hydroxyl groups), 1645 (conjugated γ-Pyrone), 1600 and 1570 cm^{-1} (benzene rings). Alkali fusion of I yielded phloroglucinol, protocatechuic acid and p-hydroxybenzoic acid[a]. By methylation with dimethyl-sulfate or diazomethane, I gave a heptamethyl ether (IV), which was negative for the ferric chloride test. Its mass spectrum showed a molecular ion peak at m/e 654 and prominent peaks at m/e 547 $(C_{24}H_8(OMe)_6 \cdot O_3 \cdot OH)^+$, m/e 474 $(C_{23}H_{11} \cdot (OMe)_5 \cdot O_2)^+$, m/e 311 $(C_{14}H_7 \cdot (OMe)_3 \cdot O \cdot CO)^+$, m/e 181 $(C_6H_2 \cdot (OMe)_2 \cdot OH \cdot CO)^+$, m/e 165 $(C_6H_3 \cdot (OMe)_2 \cdot CO)^+$ and m/e 121 $(C_6H_4 \cdot OMe \cdot CH_2)^+$.

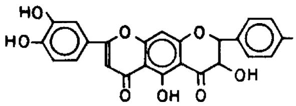
Hydrolysis of IV with boiling 15% ethanolic potassium hydroxide gave veratric acid,[a],[b], anisaldehyde[a] and a crystalline compound (V)[a], m.p. 209-210°, $C_{29}H_{30}O_{11}$, which was positive for the ferric chloride test but negative for the magnesium-hydrochloric acid test. NMR spectrum[a] (60 Mc. in $CDCl_3$) of V revealed the presence of six methoxyl groups (τ 6.05 to τ 6.42), two uncoupled methylene groups (τ 5.48 and τ 5.70), six aromatic protons (τ 2.20 to τ 4.07), and two hydroxyl groups (τ -3.80 and τ -3.66). Mass spectrum[a] of V showed a molecular ion peak at m/e 554 and prominent peaks at m/e 181 $(C_6H_2\cdot(OMe)_2\cdot OH\cdot CO)^+$, m/e 165 $(C_6H_3\cdot(OMe)_2\cdot CO)^+$ and m/e 154 $(C_6H_3\cdot(OMe)_2\cdot OH)^+$. From these data V was suggested to be a β-diketone compound. Cyclization of V[a] with boiling 2.5% methanolic sulfuric acid gave a crystalline compound (VI), m.p. 220-221°, $C_{29}H_{28}O_{10}$, which colored green with ferric chloride and red with magnesium-hydrochloric acid. NMR spectrum[a] ($CDCl_3$) of VI showed the presence of six methoxyl groups (τ 5.98 to τ 6.40), an uncoupled methylene group (τ 5.41), six aromatic and one olefinic protons (τ 2.50 to τ 4.05), and one hydroxyl group (τ -3.71). The NMR spectrum is in agreement with the structure VI.

NMR spectrum (acetone-d_6) of I showed several signals in the region between τ 2.0 and τ 6.0. A pair of doublets (J=12 cps) at τ 4.12 and τ 4.99 were assigned to C-2 and C-3 protons of ring C. Two hydrogen-bonded hydroxyl groups showed singlets at τ -3.10 and τ -2.30, and five hydroxyl groups showed broad signals at τ 0.95. NMR spectrum (acetone-d_6) of IV showed signals due to seven methoxyl groups between τ 6.07 and τ 6.34 and a pair of doublets (J=12 cps) at τ 4.10 and τ 5.08 due to C-2 and C-3 protons of ring C. Aromatic protons showed signals between τ 2.3 and τ 3.9. Protons of ring E give rise to double doublets at τ 2.52 (J=2.5, 8.5 cps), a doublet (J=2.5 cps) at τ 2.67 and a doublet (J=8.5 cps) at τ 3.08, these signals together forming an ABC pattern. The C-2' and C-6' protons, and C-3' and C-5' protons of ring B give rise to two doublets (J=9 cps) at τ 2.75 and τ 3.30, respectively. Only three protons showed signals at the higher field. Two of them appeared as <u>meta</u>-coupled doublets (J=2.5 cps) at τ 3.69 and τ 3.78, which were assigned to C-8 and C-6 proton of ring A respectively, whereas another proton give rise to a singlet at τ 3.59 due to C-6 proton of ring D. A singlet at τ 3.45 can be assigned to C-3 proton of ring F.

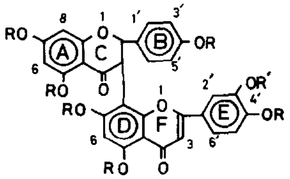
It has been suggested[4] that the bulky substituent (ring C) is not at C-6 but at C-8 of ring D in biflavonyls, if the hydroxyl group at C-5 of ring D is not exceptionally difficult to methylate. Therefore, we can suggest that C-3 of ring C is linked to C-8 of ring D. Additional evidence[a] for this suggestion is provided by the NMR spectra of IV, V, and VI. Signals due to C-8 and C-6 protons of ring A appeared as doublets (J=2.5 cps) at τ 3.69 and τ 3.78 in IV, at τ3.93 and τ4.04 in V, and at τ3.90 and τ4.00 in VI. The corresponding signals in IV, V and VI are very similar to each orther and may be assigned to C-8 and C-6 protons of ring A, respectively. In the NMR spectrum of V, the signal due to C-6 proton of ring D and that due to C-6 proton of ring A overlap at the same position (τ4.07), suggesting the linkage between C-3 of ring C and C-8 of ring D. From these data we propose for (±)-fukugetin the structure I. Hydrolysis of IV[b)] with 5% ethanolic KOH under mild conditions gave an isomer, $C_{37}H_{34}O_{11}$, m.p. 134-136°, which showed color reactions characteristic of hydroxychalcones and flavones[5)]. Acetylation of the isomer with acetic anhydride and pyridine gave monoacetate VII, $C_{39}H_{36}O_{12}$, m.p. 158-159°. The NMR spectrum of VII[b)] showed the presence of an acetoxy group (τ=7.95) and seven methoxy groups (τ=6.55-5.95). The results support structure VII for the acetate derived from the structure I for (±)-fukugetin.

Recently, Karanjgaokar et al.[6)] have obtained morelloflavone, to which they assigned the structure I. The UV spectrum and TLC of (±)-fukugetin (I) were identical with those of morelloflavone, and (±)-heptamethyl fukugetin (IV) was identical with heptamethyl morelloflavone[6)] (mixed m.p. and IR, NMR, and mass spectra)[b)]. The result indicates that (±)-fukugetin is identical with morelloflavone.

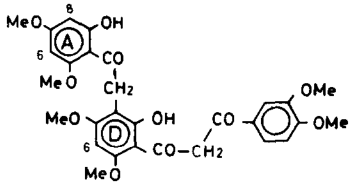




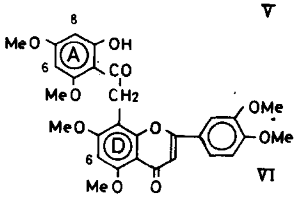
Ic



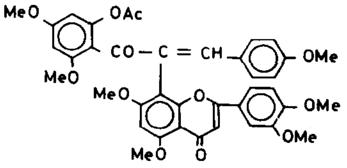
I, II : R = R′ = H
III : R = H, R′=Me
IV : R = R′ = Me



V



VI



VII

(+)-Fukugetin (II), $C_{30}H_{20}O_{11}$, showed the following ORD data: ($c=0.3$, MeOH) $[\alpha]^{29}$ (mμ) + 160 (650), + 170 (589) and + 880 (410). Its UV and NMR spectra were identical with those of I. But its IR spectrum (nujol mull) was different from that of I. (+)-Fukugetin (II) when refluxed with HBr in acetic acid changed to (±)-fukugetin (I) and when methylated with dimethyl sulfate gave IV.[b] It is noteworthy that fukugetin was isolated in an optically active form from the bark. The large coupling constants ($J=12$ cps) of C-2 and C-3 protons in ring C of I and II indicate that the hydrogens have a trans-diaxial arrangement. Studies on the absolute configuration of ring C are in progress.[a]

(±)-3'-O-methyl fukugetin (III), $C_{31}H_{22}O_{11}$, showed a very similar NMR spectrum (acetone-d_6) as that of I (=II) except the presence of a signal due to a methoxyl group (τ 6.08)[a] which was further confirmed from NMR spectrum (a siglet at τ6.21) of the acetate, m.p. 178-180°(dec.)[b]. By methylation with dimethyl sulfate, III gave IV. Alkaline hydrolysis of III yielded acetovanillon, phloroglucinol and p-hydroxybenzaldehyde.[a] From these data the structure of III was confirmed as 3'-O-methyl ether of I.

Fukugetin, m.p. 288-289°, which was isolated by the method previously reported[2,7] was shown by TLC to be I contaminated by III. Garcinin[8], which was also reported to be isolated from G. spicata Hook. f. together with fukugetin and to be considered as impure fukugetin, is most probably to be mainly (+)-fukugetin (II).

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8. Reported m.p.: 245° (ref. 7).