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FUKUGISIDE, THE FIRST BIFLAVONOID GLYCOSIDE FROM GARCINIA SPICATA HOOK. f.

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Garcinia spicata HooK. f. (Guttiferae)--Japanese name: fukugi-- is an ever-green tree commonly found in Southeast Asia, especially in Ryukyu Islands where its bark has been used as a raw material of yellow dye. In the previous paper,[1] we reported the structures of (±)-fukugetin (I), (+)-fukugetin (II) and (±)-3"'-O-methyl fukugetin (III) which were isolated from the bark of Garcinia spicata. We further studied on the constituents of fresh bark of this plant and isolated a new biflavonoid glycoside, fukugiside, which is the first example of the glycoside of biflavonoid compound. This communication deals with the structural elucidation of fukugiside (IV).

Fukugiside (IV), $C_{36}H_{30}O_{16} \cdot 2H_2O$, m.p. 242-243° (decomp.), fine yellow needles from acetone-benzene (2 : 1), showed the following ORD data: (c =0.31, MeOH) $[\alpha]^{15}$ (mμ) + 116 (650), + 155 (589), + 400 (450) and + 1258 (398). It gave brownish-green colour with $FeCl_3$ and deep red colour with Mg-HCl. The spot test on a filter paper and polyamide thin-layer chromatography plate gave fine yellow colour with 5% potassium hydroxide and gave bright yellow fluorescence under the ultraviolet light. It UV absorption maxima in ethanol at 225 (shoulder), 255 (shoulder), 274, 292 and 336mμ were very similar to those of I and the maxima showed the charactristic bathochromic shift of 5,7-dihydroxy and 3',4'-ortho-dihydroxy systems[2,3] on addition of sodium acetate, $AlCl_3$ and boric acid-sodium acetate. The IR absorption (nujol mull) appears at 3250 (hydroxyl groups), 1640 (conjugated γ-pyron), 1600 and 1570 cm^{-1} (benzene rings).

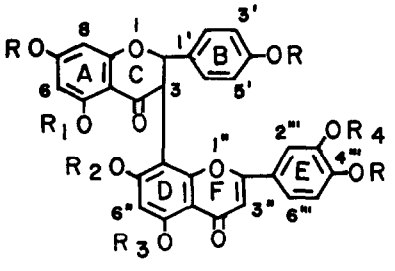
Hydrolysis of IV with sulfuric acid or cation-exchange resin (Amberlite IR-120, H-form)[4] afforded, along with D-glucose, a yellow crystalline compound, which was identified with (±)-fukugetin (I) (mixed m.p., IR and NMR spectra). The methyl ether of this compound was also identified with an authentic sample of

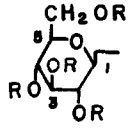
heptamethyl fukugetin (V). D-Glucose was identified by paper (PPC) and thin-layer chromatography (TLC) (microcrystalline cellulose powder) and PPC, TLC and m.p. of its osazone. On acetylation IV gave a decaacetate (VI), which was negative for the ferric chloride test. NMR spectrum (60 mc. in $CDCl_3$, τ scale) of VI revealed the presence of ten acetoxyl groups (7.55 to 8.12) and C-1 to C-6 protons of the glucose moiety (multiplets, 4.50 to 6.50). The NMR spectrum (acetone-d_6) of IV showed several signals in the region between 2.40 and 7.0 (2.0 and 6.3 in pyridine-d_5), two hydrogen-bonded hydroxyl groups showed broad singlets at -3.03 and -2.13, other hydroxyl groups showed broad signals at 0.95, and all the glucose protons showed broad signals at 4.7 - 6.6 (4.3 - 6.2 in pyridine-d_5).

In order to determine the position of glucose in the molecule, IV was methylated with methyl iodide and silver oxide in dimethylformamide (Kuhn's method)[5] at room temperature to give permethyl ether (VII), colourless syrup, which was negative for the ferric chloride test. The acid hydrolysis of VII yielded crystalline compound (VIII), m.p. 218-219°, $C_{36}H_{32}O_{11}$, which was positive for the ferric chloride test. Its UV spectrum was identical with that of V and the maxima showed the charactristic bathochromic shift of 7-hydroxy system on addition of sodium acetate. Acetylation of VIII with acetic anhydride and pyridine gave a monoacetate (IX), which was negative for the ferric chloride test. NMR spectrum of IX showed the presence of one acetoxyl group (7.82).

The NMR spectrum (acetone-d_6) of VIII showed signals due to six methoxyl groups between 6.05 and 6.35 and a pair of doublets (J=12 cps) at 4.01 and 5.05 due to C-2 and C-3 protons. Protons of ring E gave rise to double doublets at 2.51 (J=2.5, 8.5 cps), a doublet (J =2.5 cps) at 2.64 and a doublet (J =8.5 cps) at 3.05. The C-2' and C-6' protons, and C-3' and C-5'protons gave rise to two doublets (J =9 cps) at 2.75 and 3.31, respectively. The remaining three aromatic protons showed signals in the region of 3.53 - 3.84 of the spectrum. Two of them appeared as meta-coupled doublets (J =2.5 cps) at 3.70 and 3.79, which were assigned to C-8 and C-6 protons respectively, whereas another proton showed a singlet at 3.55 due to C-6" proton. A singlet at 3.51 can be assigned to C-3" proton. From these data VIII was suggested to be a 5,7,4',5",3"',4"'-hexa-O-methyl fukugetin. Additional evidence for this suggestion is provided by the

NMR spectra of V, VII, VIII, 5,7,4',7",3"',4"' -hexa-O-methyl fukugetin (X), 7,4', 5",7",3"',4"' -hexa-O-methyl fukugetin (XI) and 7,4',7",3"',4"' -penta-O-methyl fukugetin (XII)*. Signals due to C-8 and C-6 protons of ring A appeared as doublets (J =2.5 cps) at 3.69 and 3.78 in V, at 3.70 and 3.79 in VII and VIII, at 3.68 and 3.77 in X, at 3.85 (singlet, 3H: C-8, C-6 and C-6") in XI, and at 3.88 (singlet, 3H: C-8, C-6 and C-6") in XII. The corresponding signals in V, VII, VIII and X are very similar to each other and may be assigned to C-8 and C-6 protons of ring A, respectively. The NMR spectrum is in agreement with the structure VIII. NMR spectrum (acetone-d_6) of VII showed signals due to ten methoxyl groups between 6.08 and 6.60 and multiplets at 4.92 and 5.25 - 6.68 due to C-1 and C-2 to C-6 protons of glucose. Aromatic and aliphatic protons were identical with those of heptamethyl fukugetin (V). Hydrolysis of IX with boiling ethnolic potassium hydroxide gave veratric acid and anisaldehyde.





β-D-glc (R =H, Ac, Me)

I,II : R =R_1 =R_2 =R_3 =R_4 =H

III : R =R_1 =R_2 =R_3 =H, R_4 =Me

IV : R =R_1 =R_3 =R_4 =H, R_2 =β-D-glc

V : R =R_1 =R_2 =R_3 =R_4 =Me

VI : R =R_1 =R_3 =R_4 =Ac, R_2 =β-D-glc(Ac)

VII : R =R_1 =R_3 =R_4 =Me, R_2 =β-D-glc(Me)

VIII : R =R_1 =R_3 =R_4 =Me, R_2 =H

IX : R =R_1 =R_3 =R_4 =Me, R_2 =Ac

X : R =R_1 =R_2 =R_4 =Me, R_3 =H

XI : R =R_2 =R_3 =R_4 =Me, R_1 =H

XII : R =R_2 =R_4 =Me, R_1 =R_3 =H

The enzymatic hydrolysis of IV with emulsin (Sigma Chemical Co.) gave II and D-glucose to prove the β-linkage between glucose moiety and aglycone. From these data we propose for fukugiside the structure IV (=fukugetin-7"-β-glucoside).

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* Methylation of (+)-fukugetin (II) with dimethyl-sulfate and potassium hydroxide afforded V, m.p. 209-210°, X, m.p. 230-231°, XI, m.p. 181-182°, and XII, m.p. 149-150°.

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