3 N HCl (5 µl) and the mixture ether-extracted to give the acid fraction. The H_2O-soluble glycosides, HG-1′, 2′ and 3′ were isolated by PC for further study.

Attempted identification of acid. The acid fraction was co-chromatographed with a wide range of organic acids on cellulose, polyamide and Si gel TLC. Solvents used were (A) *n*-BuOH–formic A–H_2O, 10:1:5 (cellulose); (B) EtOH–0.88 NH_4OH, 95:5 (cellulose); (C) MeCN–EtOAc–HCO_2H, 81:8: 9.1:9.1 (polyamide) and (D) EtOAc–toluene, 1:1 (Si gel). Detecting methods: UV, 2% bromocresol green spray (Me_2CO–H_2O) and diazotized sulphanilic acid–Na_2CO_3 spray. By a combination of these procedures (and MS), *p*-hydroxybenzoic acid was identified. The major acid produced, co-chromatographed only with succinic acid out of all the 60 naturally occurring acids for which R_f values have been listed by Carles *et al.* [16] for solvents A and B. Confirmatory evidence however was not obtained.

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NEW ISOFLAVONE GLYCOSIDES FROM THE WOODS OF *SOPHORA JAPONICA*

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Key Word Index—*Sophora japonica*; Leguminosae; biochanin A 7-β-D-gentiobioside; biochanin A 7-β-D-xylosylglucoside.

The flavonoids of the rhizomes and the fruits of *Sophora japonica* [1–3] have been well studied but less is known of those in the wood. We have isolated biochanin A, irisolidone, maackiain, pratensein, sissotrin, irisolidone 74*O*-glucoside and rutin [4–6] and now report two new isoflavone glycosides. These were purified by the droplet counter-current chromatography (DCC) [7, 8].

The first glycoside, mp 224–226° afforded on acid hydrolysis biochanin A and glucose. From the UV spectrum it was assumed to be a 7-glucoside [9]. Exhaustive methylation by Kuhn's method gave the methylated product, which showed nine *O*-methyls and the molecular ion peak at *m/e* 720, the typical permethylated diglucose fragment ion *m/e* 423 in the ms. Methanolysis products of the permethyl ether were methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, and methyl 2,3,4-tri-*O*-methylglucopyranoside. The PMR spectrum of the glycoside shows the presence of two anomeric protons on the β-linkage as judged from the J values (δ, 4.62, *d*, J = 10Hz, 4.84, *d*, J = 6Hz). Thus, it is 7-*O*-[β-D-glucopyranosyl(1-6)-β-D-glucopyranosyl]-biochanin A.

The second glycoside, mp 228–230°, afforded on acid hydrolysis biochanin-A and glucose and xylose and had similar spectral properties to the first compound. Methanolysis of its permethylether gave methyl 2,3,4-tri-*O*-methylglucopyranoside and methyl 2,3,4-tri-*O*-methylxylopyranoside, thus showing that xylose is attached to the 6-position of glucose. The glycosidic bonds are β-configuration by PMR (δ 4.90, *d*, J = 10Hz, 5.60, *d*, J = 8Hz, respectively). Thus, it is biochanin A 7-(xylosyl(β1 → 6)glucoside).

EXPERIMENTAL

All mps were uncorrected. PMR spectra were taken at 100 MHz in $CDCl_3$ and/or d_5-Pyridine solution and chemical shifts are given in δ (ppm) scale. GLC was run with flame ionization detector using a glass column. PPC was conducted on Toyo Roshi No. 50 using the upper layer of *n*-BuOH–(C_5H_5N–H_2O (6:2:3) and aniline hydrogen phthalate as spray reagent. TLC was performed on Kieselgel G (Merck) using (a) $CHCl_3$–MeOH–H_2O (35:65:40) (lower layer), (b) C_6H_6–Me_2CO (4:1), (c) EtOAc–MeCOEt–HCO_2H–H_2O–C_6H_6 (4:3:1:1:2) (upper layer).

Isolation. Powdered woods of *Sophora japonica* (880g) collected at the Botanic Garden of Nagoya City University were extracted with MeOH for 3 hr on reflux. The MeOH extract was concentrated and partitioned between BuOH and H_2O. The BuOH layer was concentrated and precipitated with Et_2O.

Precipitate (11 g) was distributed on DCC using lower layer of $CHCl_3$–MeOH–H_2O (35:65:40) as a stationary phase and upper layer as a moving phase. Two new compounds were obtained as crystals. (2: 2.0 g, 3; 0.5 g). Sissotrin, irisolidone-7-*O*-glucoside and rutin were identified by the comparison of IR (KBr) and PMR spectrum with authentic samples. The gentiobioside was obtained as colourless prisms (MeOH–H_2O), mp 224–226°, $[\alpha]_D^{24} - 38.2°$ (C = 1.79 in MeOH–H_2O 4:1) Anal. Calcd. for $C_{28}H_{32}O_{15}$; C, 55.26; H, 5.26. Found: C, 55.15; H, 5.24 UV λ_{max}^{MeOH} nm (log ε); 262 (4.61), 324 (3.61), $\lambda_{max}^{MeOH+AlCl_3}$ nm 272, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm 272, $\lambda_{max}^{MeOH+NaOAc}$ nm 262. PMR (d_5-Py); 3.70 (3H, *s*, —OMe), 4.20–4.00 (19H, *m*), 4.62 (1H, *d*, *J* = 10Hz), 4.84 (1H, *d*, *J* = 6), 6.66 (1H, *d*, *J* = 2, H-6), 6.94 (1H, *d*, *J* = 2, H-8), 6.98 (2H, *d*, *J* = 8, H-3′,5′), 7.56 (2H, *q*, *J* = 2,8,H-2′,6′), 8.06 (1H, *s*, H-2), 13.30 (1H, —OH). Permethyl ether was obtained as colourless needles, mp 78.5–80.5°. MS M^+ 720, *m/e* 423 (6.5%), 298 (13.9%), 219 (1.7%), 187 (100%). PMR (δ ppm in $CDCl_3$); 3.36, 3, 46, 3.48, 3.50 (2 × OMe), 3.68 (2 × OMe), 3.84, 3.96, 4.24 (1H, *d*, *J* = 8Hz), 4.96 (1H, *d*, *J* = 8), 6.44 (1H, *d*, *J* = 3), 6.73 (1H, *d*, *J* = 3), 6.92 (2H, *d*, *J* = 10), 7.47 (2H, *d*, *J* = 10), 7.76 (1H, *s*). The octa-acetate crystallized as needles, mp 253–255°, PMR (δ ppm in $CDCl_3$) 1.90, 1.96, 2.02 (2 × —OCOMe), 2.06 (3 × —OCOMe), 2.40 (Arom. —OCOMe), 3.82 (OMe), 4.28 (1H, *d*, *J* = 8Hz), 4.62 (1H, *d*, *J* = 8), 5.0–5.3 (12H, *m*), 6.66 (1H, *d*, *J* = 2) 6.92 (2H, *d*, *J* = 8), 7.08 (1H, *d*, *J* = 2), 7.44 (2H, *d*, *J* = 8), 7.98 (1H, *s*). The 7-xylosylglucoside was isolated as needles (MeOH–H_2O) mp 228–230°, $[\alpha]_D^{24} - 79.5°$ (*c* = 0.52 in MeOH–H_2O 4:1). Anal. Calcd. for $C_{27}H_{30}O_{14}\cdot H_2O$; C, 54.36 H, 5.41. Found; C, 54.15 H, 5.22 UV λ_{max}^{MeOH} nm (log ε) 263 (4.55), 324 (3.56) $\lambda_{max}^{MeOH+NaOAc}$ nm 263, $\lambda_{max}^{MeOH+AlCl_3}$ nm 272, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm 273. PMR (δ ppm in d_5-pyridine) 3.72 (3H, *s*, —OMe), 4.40–4.10 (17H, *m*), 4.76 (1H, *d*, *J* = 7Hz), 4.90 (1H, *d*, *J* = 10), 6.79 (1H, *d*, *J* = 2, H-6), 7.02 (1H, *d*, *J* = 2, H-8), 7.04 (2H, *d*, *J* = 9, H-3′,5′), 7.60 (2H, *d*, *J* = 9, H-2′,6′), 8.10 (1H, *s*, H-2). Permethyl ether, needles mmp 154–156.5°. MS M^+ 676, *m/e* 379, 298, 175, 143. PMR (δ ppm in $CDCl_3$) 3.46, 3.48, 3.60 (2 × —OMe), 3.68 (2 × OMe), 3.84, 3.95, 4.22 (1H, *d*, *J* = 7), 4.87 (1H, *d*, *J* = 10), 4.94 (11H, *m*), 6.48 (1H, *d*, *J* = 2, H-6), 6.68 (1H, *d*, *J* = 2, H-8), 6.94 (2H, *d*, *J* = 8, H-3′5′), 7.50 (2H, *d*, *J* = 8, H-2′,6′), 7.80 (1H, *s*, H-2). Acetate was obtained as needles, mp 143–145°. PMR (δ ppm in $CDCl_3$) 1.92, 2.04 (3 × —OCOMe), 2.08 (2 × —OCOMe), 2.40 (Arom. —OCOMe), 3.84 (OMe), 4.56 (1H, *d*, *J* = 8Hz), 4.96 (1H, *d*, *J* = 7), 5.0–5.3 (11H, *m*), 6.64 (1H, *d*, *J* = 2), 6.94 (2H, *d*, *J* = 8), 7.00 (1H, *d*, *J* = 2), 7.44 (2H, *d*, *J* = 8), 7.94 (1H, *s*).

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A NEW TETRAMETHYLURIC ACID FROM *COFFEA LIBERICA* AND *C. DEWEVREI*

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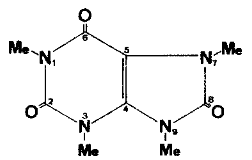
Key Word Index—*Coffea*; Rubiaceae; leaves, *O*(2),1,7,9-tetramethyluric acid.



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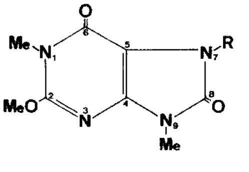

2 R = H 3 R = Me

In an earlier communication [1], we reported the presence of 1,3,7,9-tetramethyluric acid (**1**) and *O*(2),1,9-trimethyluric acid (**2**) in the genus *Coffea*. In a detailed study on the distribution of caffeine and these methylated uric acids during vegetative development of *C. liberica* [2], we noticed that, at a certain developmental stage of the plant, the leaves contain a third uric acid in concentrations mostly less than 0.1%. Based upon its transient occurrence which coincides with a decrease in concentration of (**1**) and an increase of (**2**), we supposed that it could be the metabolic intermediate and may have the structure of (**3**). The chromatographic comparison with an authentic sample of (**3**), which was synthesized earlier for proper identification of (**2**), confirmed our suggestion. We isolated the substance in pure form for identification from *C. liberica* Bull ex Hiern as well as from two varieties of *C.*