Phytochemistry, 1976, Vol 15, pp. 1184–1185. Pergamon Press Printed in England.

THE SYNTHESIS OF FLAVONOL 3-*O*-β-GENTIOTRIOSIDES

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(*Received* 10 *February* 1976)

Key Word Index—*Primula sinensis*; Primulaceae; kaempferol and quercetin 3-gentiotriosides; synthesis.

Flavonoid glycosides containing trisaccharides are not uncommon as plant constituents [1]. While the position of the sugar–aglycone linkage has usually been established, information about the structure of the trisaccharide moiety is usually incomplete. Often, only the composition, sometimes the sequence of the monosaccharides has been reported, but only in few cases has the complete elucidation of both constitution and configuration of the trisaccharide been carried out [2].

Since no synthesis of a flavonoid triglycoside has been realised, we wished to accomplish such a synthesis. For this purpose the 3-*O*-gentiotriosides of quercetin and kaempferol were selected, substances which were reported by Harborne and Sherratt in 1961 to occur in the petals of *Primula sinensis* [3]. Tentative identification of the trisaccharide as gentiotriose (*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl-(1 → 6)-D-glucose) was based mainly on partial hydrolysis affording glucose and gentiobiose. The position of the aglycone-sugar linkage was determined by methylation experiments and UV spectroscopy [3].

For the synthesis of the quercetin analogue, 7,4′-di-*O*-benzylquercetin [4] was coupled with acetobromogentiotriose [5]. It has been demonstrated repeatedly [6] that coupling of acetohalogenosugars to this aglycone involves the C_3-OH. Chromatographic separation from the unreacted aglycone was carried out either after saponification or preferably after acetylation and afforded the dibenzyl ether and its dodecacetate resp. Debenzylation and saponification (of the acetate) yielded crystalline quercetin-3-*O*-β-gentiotrioside. An analogous sequence of reactions starting from 7,4′-di-*O*-benzylkaempferol [7] gave crystalline kaempferol-3-*O*-β-gentiotrioside.

The identity of synthetic quercetin 3-gentiotrioside with the quercetin-3-*O*-triglucoside from *P. sinensis* could not be established since no mp has been reported for the latter and a reference sample was no longer available. However, the synthetic kaempferol derivative was found to be different (see Experimental) from natural kaempferol 3-triglucoside from *Primula sinensis*, indicating clearly that although it has a very closely similar trisaccharide moiety, it is not in fact the 3-gentiotrioside. Further work is, therefore, now needed on the sugar of the *Primula* glycosides.

EXPERIMENTAL

5,3′-Diacetoxy-7,4′-dibenzyloxy-3-hydroxyflavone-3-O-[*deca*-O-*acetyl*-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranoside*]. To a solution of 7,4′-dibenzyloxy-3,5,3′-trihydroxyflavone [4] (1.8 g) in quinoline (20 ml) at 0° first Drierite (3.0 g) and Ag_2O (1.34 g) and then a solution of α-acetobromogentiotriose [5] (2.3 g) in quinoline (10 ml) was added. After stirring for 3 hr at room temp. the mixture was diluted with $CHCl_3$ (100 ml), filtered and extracted with 5% H_2SO_4 (10 × 50 ml) and washed with H_2O (5 × 50 ml). After evaporation the residue was chromatographed on Si gel in C_6H_6–EtOH (9:1) to eliminate the unchanged aglycone. The crude glycoside was acetylated with C_5H_5N–Ac_2O and reprecipitated from $CHCl_3$ with EtOH to give a colorless amorphous powder (2.1 g, 39%), mp 115–127°. *Anal.* Calc. for $C_{71}H_{76}O_{34}$: C, 58.02; H, 5.19. Found: C, 57.65; H, 5.00.

7,4′-Dibenzyloxy-3,5,3′-trihydroxyflavone-3-O-[β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranoside*]. A solution of the dodecaacetate (200 mg) in MeOH (10 ml) was treated with 0.1 M NaOMe (0.6 ml) at room temp. for 48 hr, acidified to pH 6 with Amberlite IR 120. After filtration and evaporation the residue was first crystallised from Me_2CO and then from MeOH–Me_2CO (4:1) to give yellow plates (110 mg, 82%), mp 150–153°. *Anal.* Calc. for $C_{47}H_{52}O_{22}$: C, 58.09; H, 5.44. Found: C, 57.96; H, 5.83.

3-Hydroxy-5,7,3′,4′-tetraacetoxyflavone-3-O-[*deca*-O-*acetyl*-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosid*]. Debenzylation of the dodecaacetate (1.6 g) by standard catalytic hydrogenation in EtOH (20 ml) and subsequent acetylation gave after reprecipitation from EtOH–Me_2CO (9:1) an amorphous powder (0.56 g, 38%) of mp 127–137°. *Anal.* Calc. for. $C_{61}H_{68}O_{36}$: C, 53.27; H, 4.99. Found: C, 52.45; H, 4.76.

3,5,7,3′,4′-Pentahydroxyflavone-3-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranoside*]. Saponification of the tetraacetate (0.35 g) as described for the kaempferol analogue, recrystallisation of the crude product (0.10 g, 54%) first from Me_2CO and then from Me_2CO-H_2O (4:1) and MeOH–Me_2CO gave yellow platelets (50 mg) of quercetin-3-gentiotrioside mp 196–199°, $[\alpha]_D^{20}$ −47° (*c* 0.25, dimethylformamide). *Anal.* Calc. for $C_{33}H_{40}O_{22}.5H_2O$: C, 45.10; H, 5.73; H_2O, 10.25. Found: C, 44.88; H, 5.85; H_2O, 10.8.

5-Acetoxy-7,4′-dibenzyloxy-3-hydroxyflavone-3-O-[*deca*-O-*acetyl*-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranoside*]. Coupling of 7,4′-dibenzyloxy-3,5-dihydroxyflavone [7] (1.3 g) with acetobromogentiotriose as described with the quercetin analogue yielded after chromatography an amorphous decaacetate (200 mg), which was then acetylated to give after recrystallisation from ethanol a colourless amorphous powder, mp 115–120°. *Anal.* Calc. for $C_{69}H_{74}O_{32}$: C, 58.50; H, 5.27. Found: C, 57.78; H, 5.91.

3,4′,5,7-Tetrahydroxyflavone-3-O-[β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranosyl*-(1→6)-O-β-D-*glucopyranoside*]. Debenzylation by catalytic hydrogenation of the above undecaacetate (0.16 g) in ethanol and saponifi-

cation, crystallisation first from methanol and then from *n*-butanol saturated with H_2O gave light yellow microneedles of kaempferol 3-gentiotrioside which transformed at 198–200° to a viscous melt becoming clear and liquid at 230°. $[\alpha]_D^{21}$ −27° (*c* 0.13, dimethylformamide). *Anal.* Calc. for $C_{38}H_{40}O_{21}.4H_2O$: C, 46.93; H, 5.73; H_2O 8.53. Found: C, 47.11; H, 6.00; H_2O 9.5.

Comparison of natural Primula 3-*triglucoside and synthetic* 3-*gentiotrioside of kaempferol.* The natural and synthetic glycosides had identical R_f values in *n*-BUOH–HOAc–H_2O (4:1:5) (0.24, 0.24) in *n*-BuOH–EtOH–H_2O (4:1:2.2) (0.22, 0.22), in PhOH–H_2. (0.28, 0.30), and in H_2O (0.41, 0.42) but clearly separated in 5% HOAc (0.35, 0.47) and in 15% HOAc (0.51, 0.57). They had identical mobilities when electrophorized in borate buffer pH 8.8 for 3 hr at 400 V/un, H_2O_2 oxidation of the 2 glycosides gave trisaccharides with different R_G values in some solvents. R_G values for gentiobiose, the sugar from the *Primula* triglucoside and the sugar from the synthetic 3-gentiotrioside were as follows: 0.32, 0.18 and 0.16 in BAW; 0.24, 0.17 and 0.17 in BEW; 0.46, 0.24 and 0.21 in BBPW; and 0.58, 0.44 and 0.27 in PhOH–H_2O.

Acknowledgement—The authors thank Dr J. B. Harborne, University of Reading, for the comparison of natural and synthetic kaempferol 3-triglycosides.

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Phytochemistry, 1976, Vol. 15, pp 1185–1186. Pergamon Press. Printed in England.

A NEW FLAVAN GLYCOSIDE FROM *BUCKLEYA LANCEOLATA* LEAVES*

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(*Received* 14 *January* 1976)

Key Word Index—*Buckleya lanceolata*; Santalaceae; structural determination; flavan; 5,7,4′-trihydroxyflavan 5-xyloside.

Previously, Hopkins *et al.* [1] isolated acetylenic fatty acids from seeds of *Buckleya distichophylla* Torr. which grows in the eastern United States. We now report the structure of new flavan glycoside isolated from the leaves of *B. lanceolata* Miq., a species endemic to Japan.

Colour tests and UV spectrum indicated that the new glycoside (**1**) was a *para*-substituted, unconjugated phenol. MS exhibited M^+ and an aglycone peak formed by elimination of a five carbon sugar. PMR spectrum indicated the presence of four protons on a *para*-substituted benzene ring, two aromatic hydrogens giving a *meta* coupling constant, a $-CH_2CH_2-$, a CH, six –O–CH protons probably of the sugar moiety and five –OH protons. Two of the hydroxyls were phenolic and others were aliphatic, because **1** afforded the dimethyl ether **2** by reaction with CH_2N_2 and a pentaacetate **3**, containing two aromatic acetyl and three aliphatic acetyl groups. **2** was converted to a triacetate **4** by acetylation. On hydrolysis with acid or emulsin, **1** produced xylose and an aglycone **5** which gave a positive Gibbs test. On the basis of these data, **1** was presumed to be the 5-*O*-D-xyloside of 5,7,4′-trihydroxyflavan and this was confirmed by the following experiments.

Identity of the product **6** obtained from **2** by hydrolysis and authentic (2S)-7,4′-dimethoxy-5-hydroxyflavan was proved by mmp and IR comparison. The CD spectra of **5** and **6** supported the S-configuration of 2-position, because these spectra showed negative Cotton effects[2]. The coupling constant of the anomeric proton of TMSiate **7** of **1** (*J* 7.5 Hz)[3] and the result of hydrolysis of **1** using emulsin[4] indicated that xylose was bound by a β-D-glycosidic bond. **1** is thus (2S)-5,7,4′- trihydroxyflavan 5-*O*-β-D-xyloside.

*Part 1 in the series "The Chemical Components of Santalaceae".

EXPERIMENTAL

All mp's are uncorr. PMR were measured on a 100 MHz apparatus and chemical shifts are given in ppm relative to TMS as internal standard. MS were measured at 70 eV. For solvent of PC, *n*-BuOH–AcOH–H_2O (4:1:5) was used.

Plant. Plants were collected in Oume city, Tokyo in May, 1973. A voucher specimen (coll. Y. Sashida) is deposited in the Herbarium of the National Science Museum of Japan.

Extraction and isolation. Air-dried leaves (1.66 kg) were extracted with 10 l. hot MeOH for 100 hr, and the extract, after removal of solvent was extracted with *n*-hexane and subsequently with EtOAc. EtOAc extract (180 g) was fractionated by column chromatography over Si gel with CH_2Cl_2–MeOH. Fractions which were eluted with CH_2Cl_2–MeOH (9:1) gave *ca* 2.0 g colourless needles **1** after purification by re-chromatography, decolorization with active carbon and recrystallization from H_2O.

(2*S*)-5,7,4′-*trihydroxyflavan*-5-O-β-D-*xyloside* **1**. $C_{20}H_{22}O_8$ (Found: C, 61.41; H, 5.57. $C_{20}H_{22}O_8$ requires: C, 61.53; H, 5.68), mp 243°. Colour tests: benzidine, +; $FeCl_3$–K_3 $Fe(CN)_6$, +; Gibbs reag., −. $(\alpha)_D^{25°}$ −31.8°(*c* 0.40, EtOH). UV λ_{max} nm(log ε): 208(4.70), 227(shoulder, 4.38), 275(3.21), IR ν_{max}^{KBr} cm^{-1}: 3415, 1622, 1600, 1500, 1050, 834. PMR