189. Structure Elucidation and Synthesis of Flavonol Acylglycosides. III[1]). The Synthesis of Tiliroside

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Summary

Naturally occurring kaempferol 3-*O*-β-D-(6″-*O*-coumaroyl)glucopyranoside (tiliroside) has been synthesized thereby confirming its structure. This is the first acylated flavonoid glycoside to be synthesized.

Introduction. – In the plant kingdom tiliroside seems to have the widest distribution among the flavonol acylglycosides hitherto found. It was first isolated by *Hörhammer et al.* [1] from the leaves and flowers of *Tilia argentea* and has been subsequently reported to occur in *Tilia cordata* [2], *Anaphalis contorta* [3], *Helianthemum ovatum* [4], *Pinus contorta* [5], *Thymelea hirsuta* [6], *Pteridium aquilinum* [7] and some other plants.

The first structure proposed for tiliroside was a 7-*p*-coumarate of kaempferol 3-*O*-β-D-glucoside (astragalin). *Harborne* [8] showed by UV. spectroscopic investigations that tiliroside was a 3-*O*-(*p*-coumaroyl)glucoside of kaempferol. The exact position of the *p*-coumaroyl residue was shown by us to be on C(6″) of the glucose moiety on the basis of ^{13}C-NMR. spectroscopy. Thus tiliroside was assigned the structure kaempferol 3-*O*-β-D-(6″-*O*-*p*-coumaroyl)glucopyranoside [9] (**1**).

Meanwhile it was independently established that the compounds isolated from *Helianthemum ovatum* [4] and *Pteridium aquilinum* [7] were identical with tiliroside from *Tilia argentea*. The same structure was postulated for a *p*-coumarate of astragalin (tribuloside) isolated from *Tribulus terrestris* [10]. An actual comparison [4] of the samples of tiliroside and tribuloside however showed their non-identity. As a part of our program on the structure elucidation and synthesis of naturally occurring flavonol acylglycosides we undertook the synthesis of tiliroside.

[1]) Part II, see [9].

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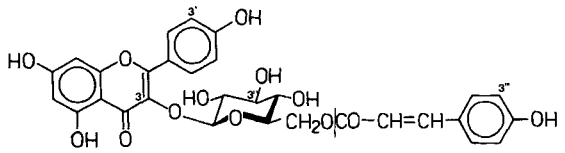

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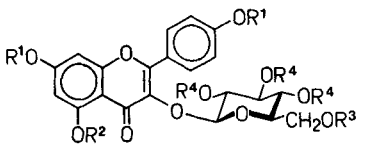

2 $R^1 = CH_2C_6H_3$; $R^2 = H$; $R^3 = COCH_2Cl$; $R^4 = OAc$
3 $R^1 = R^2 = H$; $R^3 = COCH_2Cl$; $R^4 = OAc$
4 $R^1 = R^2 = R^4 = OAc$; $R^3 = COCH_2Cl$
5 $R^1 = R^2 = R^4 = OAc$; $R^3 = H$
6 $R^1 = R^2 = R^4 = OAc$; $R^3 = p$-(*O*-Ac)coumaroyl

Results and discussion. – Condensation of 7,4′-di-*O*-benzylkaempferol [11] with 2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-*α*-D-glucopyranosylbromide[2]) in pyridine solution in presence of Ag_2CO_3 and subsequent chromatographic purification gave **2** in 54% yield. As expected, glycosidation gave the 3-*O*-*β*-D-glucosyl derivative [13]. Catalytic debenzylation of **2** and subsequent careful acetylation resulted in the product **4**. The chloroacetyl group was not affected during this sequence. The selective removal of the *O*-chloroacetyl group was effected by treatment of **4** with thiourea in methanol at RT. for 60 h yielding the compound **5**.

For coupling **5** with *p*-(*O*-acetyl)coumaroyl chloride [15], pyridine/dichloromethane was preferred. These conditions were applied by *Chittenden & Buchanan* [16] for the partial benzoylation of sugars. A long reaction time (96 h at RT.) and a large excess of the acid chloride was necessary. Higher temperature caused partial change of **5** presumably due to a C(4″) to C(6″) acetyl migration. This facile migration was even observed during attempts to purify **4** by column chromatography on silica gel. The reaction product **6** was identical with tiliroside heptaacetate [4] [7]. The crucial point of the synthesis was the removal of the acetyl groups in **6** leaving the *p*-coumaroyl moiety intact. *Zemplen* deacetylation [17] using catalytic amounts of NaOMe and the calculated quantity of MeOH was performed on 6-*O*-cinnamoylglucose tetraacetate as a model. This resulted in selective cleavage of the acetyl groups in presence of the cinnamoyl residue and the method was applied to tilirosideheptaacetate [4] [7]. Tiliroside was formed in a 67% overall yield along with minor amounts of partially deacetylated products and kaempferol 3-*O*-*β*-D-glucoside (astragalin). The purified synthetic tiliroside was identical with the natural product (mixed m.p., Rf, UV., IR., ^{1}H-NMR. and ^{13}C-NMR.) [9].

We are indebted to Dr. *L. Pijewska* and *J. Kameczki* for a sample of tiliroside, and Drs. *A. Neszmélyi, O. Seligmann* and *P. Kolonits* for NMR. spectra. Financial support enabling Dr. *B. Vermes* to stay as a visiting scientist at the Institut für Pharmazeutische Biologie, München, was kindly provided by the *Deutsche Forschungsgemeinschaft* on a contract with the *Institute for Cultural Relations*, Budapest.

[2]) The acylated glucopyranosylbromide was synthesized according to the method of *Gagnaire & Vottero* [12] starting with the acylation of *β*-D-1,2,3,4-tetra-*O*-acetylglucopyranose with chloroacetyl chloride in ether/pyridine followed by bromination with HBr/AcOH.

Experimental part

General remarks. M.p. were determined on a *Kofler* block and are uncorrected. UV. spectra (nm) were run in *a*) EtOH, *b*) with NaOAc, *c*) with $AlCl_3$, *d*) with NaOMe. IR. spectra (cm^{-1}) were recorded as KBr pellets; NMR. spectra (ppm, *J* in Hz) were recorded at 60 and 100 MHz for ^1H and 23.5 MHz for ^{13}C with TMS as internal standard. Column chromatography was performed on silica gel and TLC. on *Merck*-$6F_{254}$-plates. Solvent systems: A = toluene/EtOH 9:1; B = toluene/EtOH 9:2; C = toluene/Me_2CO 5:4; D = EtOAc/MeOH/H_2O 100:16,5:13,5.

1. *Synthesis of 7,4'-Dibenzyloxy-3,5-dihydroxyflavone-3-O-β-D-(2'',3'',4''-tri-O-acetyl-6''-O-chloroacetyl)glucopyranoside* (**2**). To a solution of 7,4'-*O*-dibenzyl kaempferol [11] (466 mg) in pyridine (10 ml), Drierite (800 mg), Ag_2CO_3 (230 mg) and *α*-D-2,3,4-tri-*O*-acetyl-6-*O*-chloroacetylglucopyranosyl bromide [12] (750 mg) were added at 0°. After stirring for 1.5 h in the dark another portion (250 mg) of the bromide was added and stirring continued for another 1.5 h. The mixture was diluted with $CHCl_3$ (80 ml), filtered, extracted with 3% aq. sulfuric acid and washed with sat. aq. $NaHCO_3$-solution and water. After evaporation of the solvent the residue was purified by column chromatography (A, Rf 0.64) to yield the glucoside **2** (450 mg, 54%) as a colorless, amorphous powder (from EtOH/Me_2CO 9:1), m.p. 84–88°. – UV. (EtOH): (*a*) 262, 325; (*b*) 262, 325; (*c*) 275, 297, 337, 398; (*d*) 284, 380. – IR.: 1610, 1640, 1745 (CO). – ^1H-NMR. ($CDCl_3$): 2.01, 2.03, 2.12 (*s*, 3 H each, 3 OAc(glucose)); 3.82 (*s*, 2 H, CH_2Cl); 4.08 (*m*, 3 H, H–C(5'',6'',6'')); 5.13 and 5.17 (*s*, 2 H each, 2 CH_2Ph); 5.18–5.40 (3 H, H–C(2'',3'',4'')); 5.62 (*d*, *J* = 8.5, 1 H, H–C(1'')); 6.46 (*d*, *J* = 2.0, 1 H, H–C(6)); 6.52 (*d*, *J* = 2.0, 1 H, H–C(8)); 7.08 (*d*, *J* = 8.5, 2 H, H–C(3',5')); 7.45 (10 H, arom.); 8.01 (*d*, *J* = 8.5, 2 H, H–C(2',6')); 12.5 (*s*, 1 H, HO–C(5)).

$C_{43}H_{39}ClO_{15}$ (831.2) Calc. C 62.13 H 4.73 Cl 4.27% Found C 62.52 H 4.44 Cl 4.25%

2. *Synthesis of 5,7,4'-Tri-O-acetyloxy-flavone-3-O-β-D-(2'',3'',4''-tri-O-acetyl-6''-O-chloroacetyl)-glucopyranoside* (**4**). By catalytic hydrogenation **2** (450 mg) was debenzylated in EtOH to yield **3** (390 mg, 90%) as a colourless powder, Rf 0.49 (B).

$C_{29}H_{27}ClO_{15}$ (651.0) Calc. C 53.50 H 4.18 Cl 5.44% Found C 53.92 H 4.35 Cl 5.74%

Compound **3** (390 mg) was immediately acetylated with acetic anhydride (2 ml) in presence of pyridine (2 ml) and gave after the usual work-up a grey precipitate which was purified by column chromatography (C, Rf 0.5, 400 mg, 88%), white needles, m.p. 196–199° (EtOH). – UV. (EtOH): (*a*) 248, 306. – IR.: 1620, 1640, 1760 (CO). – ^1H-NMR. ($CDCl_3$): 2.01 (*s*, 6 H, 2 OAc(glucose)); 2.12 (*s*, 3 H, OAc(glucose)); 2.28 (*s*, 6 H, 2 OAc(flavone)); 2.42 (*s*, 3 H, OAc(flavone)); 3.95 (*s*, 2 H, CH_2Cl); 4.10 (*m*, 3 H, H–C(5'',6'',6'')); 4.87–5.07 (*m*, 3 H, H–C(2'',3'',4'')); 5.56 (*d*, *J* = 8.0, 1 H, H–C(1'')); 6.88 (*d*, *J* = 2, 1 H, H–C(6)); 7.15–7.40 (*m*, 3 H, H–C(8,3',5')); 8.1 (*d*, *J* = 8.0, 2 H, H–C(2',6')).

$C_{35}H_{33}ClO_{18}$ (777.1) Calc. C 54.09 H 4.28 Cl 4.56% Found C 53.60 H 4.72 Cl 4.67%

3. *Synthesis of 5,7,4'-Tri-O-acetyl-flavone-3-O-β-D-(2'',3'',4''-tri-O-acetyl)glucopyranoside* (**5**). To a solution of **4** (100 mg) in methanol (18 ml), thiourea (100 mg) in MeOH (5 ml) was added. After standing at RT. for 60 h the solvent was evaporated and the residue was extracted with $CHCl_3$, filtered and washed with water. Evaporation of solvent yielded a white microcristalline material (80 mg, 90%), m.p. 118–125°; Rf 0.26 (C). This compound was pure enough for the next stage as attempts at further purification on silica gel or repeated crystallization caused partial degradation of the product. – UV. (EtOH): (*a*) 250, 310. – IR.: 1645, 1750 (CO).

$C_{33}H_{32}O_{17}$ (700.6) Calc. C 56.57 H 4.60% Found C 56.30 H 4.68%

4. *Synthesis of 3,5,7,4'-Tetrahydroxyflavone-3-O-β-D-(6''-O-p-coumaroylglucopyranoside)-hepta-acetate (tilirosideheptaacetate)* (**6**). To a solution of **5** (140 mg) and *p*-(*O*-acetyl)coumaroyl chloride [15] (160 mg) in CH_2Cl_2, pyridine (2 ml) was added at 0° and the mixture left at RT. for 96 h. During this period a further 160 mg *p*-(*O*-acetyl)coumaroyl chloride was added in 4 portions. The mixture was diluted with CH_2Cl_2 (30 ml), extracted with 3% aq. sulfuric acid and washed with sat. aq. $NaHCO_3$-solution and water. After evaporation of the solvent the residue was chromatographed (C, Rf 0.39) to yield tilirosideheptaacetate (**6**) (110 mg, 62%) as colorless needles from EtOH/$CHCl_3$ 20:1, m.p. 185–188° ([4]: 187–189). – IR. (KBr): 1620, 1700, 1760 (CO) (superimposable with that of an authentic sample). – ^1H-NMR. ($CDCl_3$): 2.01 (*s*, 6 H, 2 OAc(glucose)); 2.13 (*s*, 3 H, OAc(glucose)); 2.20 (*s*, 3 H,

OAc); 2.33 (*s*, 6 H, 2 OAc); 2.45 (*s*, 3 H, OAc); 3.65 (*m*, 1 H, H–C(5″)); 4.10 (*m*, 2 H, H–C(6″,6″)); 4.9–5.1 (*m*, 3 H, H–C(2″,3″,4″)); 5.6 (*d*, 1 H, H–C(1″)); 6.27 (*d*, $J = 13$, 1 H, –CH=); 6.80 (*d*, $J = 2$, 1 H, H–C(6)); 7.12 and 7.18 (*d*, $J = 7$, 2 H each, H–C(3′,5′,3‴,5‴)); 7.24 (*d*, $J = 2$, 1 H, H–C(8)); 7.52 (*d*, $J = 7$, 2 H, H–C(2‴,6‴)); 7.54 (*d*, $J = 13$, 1 H, –CH=); 8.01 (*d*, $J = 7$, 2 H, H–C(2′,6′)).

$C_{44}H_{40}O_{20}$ (888.8) Calc. C 59.46 H 4.54% Found C 59.10 H 4.77%

5. *Synthesis of 3,5,6,4′-Tetrahydroxyflavone-3-O-β-D-(6″-O-p-coumaroyl)glucopyranoside (tiliroside)* (**1**). To a solution of **6** (180 mg) in $CHCl_3$ (10 ml), 1N NaOMe in MeOH (0.03 ml) was added and left at RT. for 2 h. After acidification with AcOH and evaporation of the solvent the residue was purified by chromatography (D, Rf 0.47) to yield 80 mg (67%) of tiliroside as pale yellow needles from $MeOH/H_2O$ 3:2, m.p. 267–269° (undepressed on admixture with an authentic sample) ([4]: 269–271°, [1]: 247–256°); $[\alpha]_D^{24°} = +67.8$ ($c = 0.12$, MeOH); $[\alpha]_D^{24°} = +69.9$ ($c = 0.1001$, MeOH) [4]. – UV. (EtOH): (*a*) 265, 320; (*b*) 273, 320; (*c*) 275, 305, 400; (*d*) 275, 380. – ^{13}C-NMR. (DMSO-d_6, kaempferol moiety): 177.5 (C(4)); 164.1 (C(7)); 161.2 (C(5)); 159.9 (C(4′)); 156.4, 156.5 (C(9,2)); 133.1 (C(3)); 129.9, 130.7 (C(2′,6′)); 120.9 (C(1′)); 115.1, 115.8 (C(3′,5′)); 104.0 (C(10)); 98.8 (C(6)); 93.6 (C(8)). – ^{13}C-NMR. (DMSO-d_6, *p*-coumarate moiety): 166.1 (C(9‴)); 159.9 (C(4‴)); 144.4 (C(7‴)); 130.7, 129.9 (C(2‴,6‴)); 125.1 (C(1‴)); 115.1, 115.8 (C(3‴,5‴)); 113.9 (C(8‴)). – ^{13}C-NMR. (DMSO-d_6, β-D-glucopyranoside moiety): 101.3 (C(1″)); 76.5 (C(3″)); 74.3, 74.2 (C(2″,5″)); 70.2 (C(4″)); 63.0 (C(6″)).

$C_{30}H_{26}O_{13}$ (594.5) Calc. C 60.61 H 4.41% Found C 60.95 H 4.71%

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