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TERPENOIDS AND FLAVONOIDS OF *BRIDELIA FERRUGINEA**

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Key Word Index—*Bridelia ferruginea*; Euphorbiaceae; terpenoids; flavonoids; D:A-friedo-oleanan-3β-ol; D:A-friedo-oleanan-3-one; sitosterol; stigmasterol; quercetin 3-glucoside; rutin; myricetin 3-rhamnoside; myricetin 3-glucoside.

Abstract—The terpenoid and flavonoid constituents of the hitherto unexamined medicinal plant *Bridelia ferruginea* are reported. Quercetin, quercetin 3-glucoside, rutin, myricetin 3-glucoside and myricetin 3-rhamnoside were identified.

INTRODUCTION

The genus *Bridelia* (Euphorbiaceae) has received very little phytochemical attention. Triterpenoids have been reported from *B. mooni* Thw. [1]. We report here our work on the West African medicinal plant, *B. ferruginea* Benth., used extensively for a variety of ailments, as well as a source of local dyestuffs [2]. Crude extracts of this plant have been reported to lower the fasting blood sugar level in albino rats and humans [3, 4].

RESULTS AND DISCUSSION

The petrol extract of the roots yielded D:A-friedo-oleanan-3β-ol (**1**), D:A-friedo-oleanan-3-one (**2**), sitosterol (**3**), hexadecanoic acid (**4**), a phytosterol ester shown by its mass spectra and ^{1}H NMR to be mainly stigmasterol with a long-chain fatty acid component, the nature of which was not determined, and a 1:1 mixture of sitosterol and stigmasterol.

The aqueous methanol extract of the leaves mainly yielded flavonoid glycosides, the major component of which was rutin (**5**). Quercetin 3-glucoside (**6**), quercetin (**7**) and two myricetin glycosides, **8** and **9**, were also isolated.

Compound **8** had an mp of 190–192°. Its UV behaviour in methanol–sodium methoxide and methanol–sodium acetate, with aluminium chloride, aluminium chloride–hydrochloric acid, sodium acetate–boric acid, and the formation of an instant intense yellow colour with zirconium oxychloride–citric acid, indicated a possible myricetin glycoside with a free hydroxyl in position 3 [5]. The ^{1}H NMR showed the presence of rhamnose, confirmed by hydrolysis to rhamnose and myricetin. The above evidence, together with the R_f values (PC, TLC) in various solvents, suggested that the compound probably was myricetin 3′-rhamnoside. Similar examination of **9** [mp 219–221°, 245–246.5° (decomp.), UV λ_{max} nm: 256, 307 (sh), 365 (decomp.)], suggested it could be myricetin 3′-glucoside (cannabiscitrin) (lit. mp 195°, 243° (decomp.) [6]; 220°, 245° (decomp.) [7]).

However, on peracetylation, **8** gave an octa-acetate which exhibited a sharp 2-proton singlet at δ7.69 indicating symmetrically disposed H-2′ and H-6′ in ring B. This indicated that the sugar could not be in the 3′-position as suggested by the UV evidence and colour reactions. Similarly, peracetylated **9** gave a sharp 2-proton singlet at δ7.86. That the signal for H-2′ and H-6′ was more downfield in **9** than in **8**, is indicative of possible interaction of the two protons with the acetate group at the C-6″ of glucose, compared with the rhamnose in **8**, whose C-6″ is not functionalized. This interaction would be discernible if the sugars are in the 3-position in both compounds. It is significant to note that, in quercetin 3-glucoside octa-acetate, H-2′ and H-6′ occur as a multiplet centred around δ7.9–8.0. In addition, in the spectra of the peracetates of **8** and **9**, a six-proton singlet for the symmetrically disposed C3′ and C-5′ acetyl groups is observed at δ2.34 whereas, in quercetin 3-glucoside, the two acetyl groups of the B-ring occur as two separate three-proton singlets around δ2.34, thus further confirming the location of the glyosyl unit in the 3-position. Finally, a ^{13}C NMR study of **8** showed the compound to be identical with myricetin 3-rhamnoside [8, 9].

The ^{13}C NMR of the nona-acetate of **9** was also studied, albeit on a rather small amount of material. The results are in good agreement with the calculated values for the various carbon atoms (see Experimental). In view of the rather scanty lit. data on the ^{13}C NMR of myricetin glucosides and their peracetylated derivatives [8, 9], assignments were compared with data reported for faralatroside, (a kaempferol triglycoside) and its peracetate [10]. Our results provide further evidence that the sugar in **9** is in the 3-position and not in the 3′-position. The C-3′ and C-5′ atoms were observed as two equivalent carbons at δ143.3, as were C-2′ and C-6′ at δ121.5. If the 3-position had a free hydroxyl group, acetylation would have resulted in a significant upfield shift. The C-3 atom, however, was observed at δ132.6 (or 136.8) which is in the range for 3-glycosides [8]. Compound **9** was, therefore, characterized as myricetin 3-glucoside.

*Part 14 in the series "Constituents of West African Medicinal Plants". For Part 13 see, Achenbach, H., Renner, C. and Addae-Mensah, I. (1982) *Planta Med.* **46**, 88

In view of the difficulty in identifying 3- or 3′-myricetin glycosides from UV data and colour reactions alone, it is possible that the previously characterized myricetin 3′-arabinoside, 3′-glucoside and 3′-rhamnoside [6, 11–13] are, in fact, 3-glycosides and their identity should be checked.

EXPERIMENTAL

Plant material was collected from Dodowa Road in Ghana in October 1979 by Mr. A. A. Enti. Voucher specimens, Nos. 8005/8018 are deposited at the Institute of Pharmacy, Erlangen. Unless otherwise stated, NMR δ-values are in ppm, J-values in Hz and TMS was used as an int. standard. Mps are uncorr. PC was on the descending mode unless stated otherwise.

Leaves. After defatting with petrol (bp 50–70°) the ground leaves (1 kg) were extracted with MeOH. The extract was concd and successively extracted with Et_2O, $CHCl_3$, EtOAc and *n*-BuOH. The residue from the EtOAc fraction (15.8 g) was redissolved in a minimum of MeOH and reppted with Et_2O. This gave a solid (6.86 g) and the Et_2O mother liquor, on evaporation, gave a yellow crystalline residue (2.8 g), responding to a flavonoid test. Similar treatment of the *n*-BuOH fraction gave a brown residue (10.2 g) and crude flavonoids (5.8 g).

The flavonoids (3 g) were separated on a polyamide column (Macherey–Nagel, 300 g) using $CHCl_3$–MeOH (2:1, 1:1) and, finally, MeOH, to give six main fractions. Fraction 2 (1.5 g) was further rechromatographed on polyamide (150 g), using $CHCl_3$–EtOH–methylethylketone–Me_2CO (40:20:5:1), to give mainly rutin (0.75 g) which was recrystallized from aq. MeOH, mp 185–187°, $[\alpha]_D^{22}$ −36° (C_5H_5N; *c* 1). UV and ^1H NMR of the glycoside, PC and TLC (comparison with authentic sample): consistent with structure; peracetate, mp 120–122°, $[\alpha]_D^{22}$ −76° (MeOH; *c* 1). ^1H NMR: identical with lit. data.

Quercetin 3-O-β-D-glucoside. Obtained from fraction 3 by rechromatography (polyamide) as for rutin, mp 189–190°, $[\alpha]_D$ −7.5° (MeOH; *c* 1), −28.5° (C_5H_5N; *c* 1); structure determined by UV, PC, TLC and hydrolysis to glucose and quercetin (as pentamethyl ether, mp 141–144°; MS: $[M]^+$ *m/z* 374) and preparation of the octa-acetyl derivative, mp 125–127°, $[\alpha]_D$ −34.5° (MeOH; *c* 1). ^1H NMR: consistent with the structure, as well as preparation of the penta-acetate of the aglycone, mp 189–192°.

Fraction 4 contained a mixture of rutin, quercetin 3-glucoside, and two minor unidentified components.

Myricetin 3-O-rhamnoside (**8**). Further purification of fraction 5 as previously described, and recrystallization (aq. MeOH), gave yellow crystals, mp 190–192° (170 mg), $[\alpha]_D^{20}$ −123° (MeOH; *c* 1). UV λ_{max}^{MeOH} nm: 256, 310 (sh), 353; $\lambda_{max}^{MeOH+NaOMe}$ nm: 268, 321, 390 (decomp.): $\lambda_{max}^{MeOH+AlCl_3}$ nm: 230 (sh), 273, 316, 424; $\lambda_{max}^{MeOH+AlCl_3-HCl}$ nm: 228, 274, 313.5, 404; $\lambda_{max}^{MeOH+NaOAc}$ nm: 270, 323, 406 (decomp.); $\lambda_{max}^{MeOH+NaOAc-H_3BO_3}$ nm: 257, 370. ^1H NMR (DMSO-d_6, 60 MHz): δ0.9 (3H, *d*, Me of rhamnose); 5.2 (1H, *br*, rhamnose anomeric H); 6.2, 6.4 (2H, H-6, H-8); 6.9 (2H, H-2′, H-6′). $ZrOCl_2$–citric acid test positive. Hydrolysis (HCl–MeOH, 1.1, reflux 2 hr) gave myricetin (mp > 350°, hexamethyl ether, mp 148–150°; MS $[M]^+$ *m/z* 402) and rhamnose. ^{13}C NMR (DMSO-d_6, int. standard TMS, 22.5 MHz, lit. values in parentheses): 17.4 (17.8 [8], 21.4 [9], C-6″), 69.9 (70.1, C-5″), 70.4 (70.5, 70.7, C-2″, C-3″), 71.2 (71.6, C-4″), 93.4 (93.6, C-8), 98.6 (98.7, C-6), 101.9 (102.1, C-1″), 104.0 (104.2, C-10), 107.9 (108.3, C-2′, C-3′), 119.6 (119.8, C-1′), 134.2 (134.5, C-3), 136.4 (136.5, C-4′), 145.7 (145.8, C-3′, C-5′), 156.3 (156 5, C-2), 157.4 (157.4, C-9), 161.2 (161 4, C-5), 164.0 (164.1, C-7), 177.7 (177.8, C-4) [8, 9].

Myricetin 3-rhamnoside octa-acetate. The glycoside (20 mg) was acetylated with Ac_2O (0.5 ml) and C_5H_5N (0.5 ml) to give the octa-acetate as white needles, mp 118–120°, $[\alpha]_D^{20}$ −156° (MeOH; *c* 1). ^1H NMR ($CDCl_3$): δ0.92 (3H, *d*, *J* = 6 Hz, Me of rhamnose); 1.98 (3H, *s*), 2.00 (3H, *s*), 2.14 (3H, *s*) (three rhamnose acetyl groups), 2.31 (3H, *s*) 2.33 (6H, *s*), 2.35 (3H, *s*), 2.43 (3H, *s*) (five aromatic acetyl groups), 3.32 (1H, *m*, H-5″), 4.93 (1H, *dd*, $J_1 \sim J_2 \sim 10$ Hz, H-4″), 5.18 (1H, *dd*, $J_1 \sim 10$ Hz, $J_2 \sim 3$ Hz, H-3″), 5.67 (1H, *dd*, $J_1 \sim 3$ Hz, $J_2 \sim 2$ Hz, H-2″), 5.74 (1H, *d*, $J \sim 2$, anomeric proton), 6.85 (1H, *d*, $J \sim 2$ Hz), 7.30 (1H, *d*, $J \sim 2$ Hz) (H-6, H-8), 7.69 (2H, *s*, H-2′, H-6′).

Myricetin 3-O-β-D-glucoside (**9**). This was obtained from fraction 6 as the slowest running component. Rechromatography on polyamide as before gave a yellow crystalline compound (17.8 mg), sparingly soluble in MeOH and C_5H_5N, mp 219–221°, then 245–246.5° (decomp.; 252–253° after drying on P_2O_5). These physical constants are very similar to those reported in the lit. for myricetin 3′-glucoside (lit. mp 195°, then decomp. 243° [6]; 220°, then decomp. 245° [7]). UV λ_{max}^{MeOH} nm: 256, 307 (sh), 365; $\lambda_{max}^{MeOH+NaOMe}$ nm: 271, 323, 406 (decomp.); $\lambda_{max}^{MeOH+AlCl_3}$ nm: 272, 313, 424; $\lambda_{max}^{MeOH+AlCl_3-HCl}$ nm: 273, 310, 379 (sh), 410; $\lambda_{max}^{MeOH+NaOAc}$ nm: 272, 325, 406 (decomp.); $\lambda_{max}^{MeOH+H_3BO_3}$ nm: 261, 380. Hydrolysis gave myricetin and glucose. $ZrOCl_2$–citric acid test positive.

Nona-acetyl derivative. Prepared by acetylation (5 mg) as described above, to give white needles, mp 183–185°, $[\alpha]_D^{20}$ −71° (MeOH; *c* 1) (lit. mp for myricetin 3′-glucoside nona-acetate, 194° [12]). ^1H NMR ($CDCl_3$): δ1.91 (3H, *s*, glucose C-6″ acetate); 1.99 (3H, *s*), 2.02 (3H, *s*), 2.13 (3H, *s*) (three glucose acetyls), 2.32 (3H, *s*), 2.34 (9H, *s*), 2.45 (3H, *s*), (five aromatic acetyl groups), 3.62 (1H, *m*, H-5″), 3.91–4.06 (2H, *m*, H-6″), 5.05 (1H, *dd*, $J_1 \sim J_2 \sim 10$ Hz), 5.17–5.33 (2H, *m*, H-4″, H-3″, H-2″), 5.65 (1H, *d*, $J \sim 8$ Hz, anomeric proton of β-glucoside), 6.84 (1H, *d*, $J \sim 2$ Hz), 7.31 (1H, *d*, $J \sim 2$ Hz, H-6, H-8), 7.86 (2H, *s*, H-2′, H-6′). ^{13}C NMR ($CHCl_3$,* and ** signify interchangeable assignments): δ156.5 (C-2), 132.6** (C-3), 171.8 (?) (C-4), 153.3* (C-5), 113.5 (C-6), 154.1* (C-7), 108.0 (C-8), 150.3* (C-9), 118.4 (?) (C-10), 128.0 (C-1′), 121.5 (C-2′), 143.3 (C-3′), 136.8** (C-4′), 143.3 (C-5′), 121.5 (C-6′), 98.6 (C-1″), 71.5 (C-2″), 72.8 (C-3″), 68.5 (C-4″), 72.3 (C-5″), 61.5 (C-6″), 167 5, 167.8, 169.0, 169.3, 170.0, 170.5 (C=O), 20.1, 20.4, 20.6, 20.8, 21.1, 29.6 (?) (Me–C=O).

Roots. Ground root bark (3.8 kg) was extracted with petrol (bp 50–70°). The crude extract (8.6 g) was then separated into eight main fractions on a column (silica gel, petrol–$CHCl_3$ increasing in polarity, $CHCl_3$ and, finally, $CHCl_3$–MeOH increasing in polarity). Fraction 1 gave the unidentified phytosterol ester, mp 86°, and a hydrocarbon mixture which was not further examined. Prep. TLC of fraction 2 gave mainly hexadecanoic acid {mp, IR, ^1H NMR ($CDCl_3$ as solvent and int. standard δ7.26): δ0.9 (3H, *t*, Me) 1.25 [24H, $(CH_2)_{12}$], 1.62 (2H, *m*, $-CH_2-CH_2C=O$), 2.35 (2H, *t*, $J \sim 8$ Hz, $CH_2C=O$)} MS: $[M]^+$ *m/z* 256. Traces of docosanoic acid (*m/z* 340) were also detected.

D: A-Friedo-oleanan-3-one. This was separated by prep. TLC from fraction 3 (100 mg) (cyclohexane–$CHCl_3$, 2:1) and recrystallized from $CHCl_3$–petrol as white needles (20 mg), mp 260–261° (lit. 264–265° [1]), $[\alpha]_D^{22}$ −21.5° ($CHCl_3$; *c* 1; lit. −21.6° [1]). MS: $[M]^+$ *m/z* 426. IR and ^1H NMR: consistent with structure.

D: A-Friedo-oleanan-3β-ol. Fraction 4 on prep. TLC gave more friedo-oleanan-3-one and a compound, mp 279–281° (2.7 mg), $[\alpha]_D^{22}$ +23° ($CHCl_3$; *c* 1); MS: $[M]^+$ *m/z* 428. IR and ^1H NMR consistent with title compound [lit. mp 279–283°, $[\alpha]_D$ +22° ($CHCl_3$) [1]].

Fraction 5 gave mainly sitosterol (63 mg), mp 142–144°, $[\alpha]_D$ −36° ($CHCl_3$; *c* 1) (lit. −36.1° [1]), direct comparison on TLC with authentic sample, MS, ^1H NMR, IR. Further examination of the mother liquor gave a mixture (92 mg) consisting of an almost 1:1 mixture of sitosterol and stigmasterol (MS, ^1H NMR). This was not further purified. Fractions 6–8 gave

small quantities of a complex mixture with some 10–16 components (TLC) which were not further investigated.

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