Phytochemistry, Vol. 20, No. 10, pp. 2434–2435, 1981.
Printed in Great Britain.

0031-9422/81/102434–02 \$02.00/0

O(3)-(2-ACETYLAMINO-2-DEOXY-β-D-GLUCOPYRANOSYL)-OLEANOLIC ACID, A NOVEL TRITERPENOID GLYCOSIDE FROM TWO *PITHECELLOBIUM* SPECIES

HELMUT RIPPERGER, ALFRED PREISS and JÜRGEN SCHMIDT

Institute of Plant Biochemistry, Academy of Sciences of the GDR, Halle (Saale), German Democratic Republic

(*Received* 13 *March* 1981)

Key Word Index—*Pithecellobium cubense*; *Pithecellobium arboreum*; Leguminosae; triterpenoid glycoside; *O*(3)-(2-acetylamino-2-deoxy-β-D-glucopyranosyl)-oleanolic acid.

Abstract—A new triterpenoid glycoside containing an amino sugar moiety has been isolated from *Pithecellobium cubense* and *P. arboreum* and identified as *O*(3)-(2-acetylamino-2-deoxy-β-D-glucopyranosyl)-oleanolic acid. β-D-Glucopyranosyl-α-spinasterol was also obtained from *P. cubense*.

A new triterpenoid glycoside has been isolated from *Pithecellobium cubense* Bisse and *P. arboreum* (L.) Urban. While both the electron impact (EI) and the electron attachment (EA) mass spectra only showed peaks corresponding to a triterpenoid hydroxy acid of the α- or β-amyrin series [1], the spectra of the acetyl derivative in addition indicated an amino-deoxyhexosyl moiety (see Experimental). The ^{13}C NMR spectrum (Table 1) was in agreement with the structure *O*(3)-(2-acetylamino-2-deoxy-β-D-glucopyranosyl)-oleanolic acid. The IR spectrum showed the *N*-acetyl group. This structure was confirmed by hydrolysis to oleanolic acid [6] and 2-amino-2-deoxy-α-D-glucose hydrochloride [7]. The negative molecular rotation difference between the glycoside and its aglycone [6] ($\Delta[M]_D$ −75° in pyridine) proved the β-configuration of the glycoside ($[M]_D$ of methyl 2-acetylamino-2-deoxy-β-D-glucopyranoside −111° in water [7], of the α-D-isomer +308° in water [7]) [8]. This assignment was confirmed by the ^1H NMR spectrum ($J_{1',2'} = 8$ Hz). The new compound is the first natural triterpenoid glycoside containing an amino sugar moiety.

Table 1. ^{13}C NMR spectrum of *O*(3)-(2-acetylamino-2-deoxy-β-D-glucopyranosyl)-oleanolic acid (**1**)*

Carbon	δ	Carbon	δ	Carbon	δ
1	38.6	14	42.2	27	26.2
2	26.4	15	28.3	28	180.1
3	89.1	16	23.7	29	33.2
4	39.7	17	46.6	30	23.7
5	55.8	18	42.0	1′	104.8
6	18.5	19	46.5	2′	58.1
7	33.2	20	30.9	3′	76.1
8	39.2	21	34.3	4′	72.9
9	48.0	22	33.2	5′	78.2
10	37.0	23	28.2	6′	63.0
11	23.7	24	17.3	Me(Ac)	23.7
12	122.5	25	15.4	CO(Ac)	170.1
13	144.7	26	17.0		

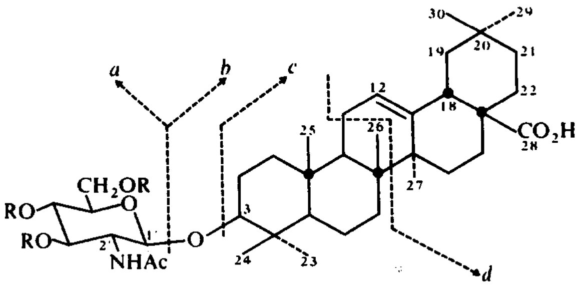
* In C_5D_5N at 50.33 MHz, in ppm, measured from the central solvent line and calculated to TMS; assignment by comparison with the chemical shifts of saikosaponin-f [2], the methyl ester of oleanolic acid [3], a 2-acetylamino-2-deoxy-β-D-glucoside [4] as well as shift differences for methyl ester → acid anion [5], in accordance with the multiplicities in the off-resonance decoupled and with the noise off-resonance decoupled spectra.

β-D-Glucopyranosyl-α-spinasterol was also isolated from *P. cubense*.

EXPERIMENTAL

Pithecellobium cubense Bisse and *P. arboreum* (L.) Urban were collected in March in Cuba, Pinar del Río, Cerca del Pan de Guajaibón, and identified by Lic. Pedro Herrera. Voucher specimens are retained in the Herbarium of the Institute of Botany, Academy of Sciences of Cuba, Havana.

O(3)-(*2-Acetylamino-2-deoxy-β-D-glucopyranosyl*)-*oleanolic acid* (**1**). Dried (40°) and ground leaves of *P. cubense* or *P. arboreum* were extracted with MeOH at room temp. Evapn *in vacuo* gave a residue which was partitioned between 0.5 N HCl and C_6H_6–Et_2O (1:1). After addition of $KHCO_3$ to the aq. layer, the latter was extracted with $CHCl_3$–EtOH (2:1). Evapn of the organic solvents gave raw material, which was chromatographed over Si gel with $CHCl_3$–MeOH (9:1). Crystallization from MeOH afforded **1**, yield 0.15% from *P. cubense* and 0.06% from *P. arboreum*, respectively, mp 282–284° (dec.), $[\alpha]_D^{20}$ +36.7° (pyridine, *c* 0.98), R_f 0.30 [Si gel; $CHCl_3$–MeOH (4:1), detection by $Ce(SO_4)_2$–H_2SO_4 at 120°]. (Found: C, 68.4; H, 9.7; N, 2.2. $C_{38}H_{61}NO_8$ requires: C, 69.2; H, 9.3; N, 2.1%). IR ν_{max}^{KBr} cm^{-1}: 1689 (CO_2H), 1660, 1550 (NHCO). ^1H NMR (200.13 MHz, C_5D_5N–D_2O, TMS): δ 0.67 (3 H, *s*, Me), 0.88 (9 H, *s*, 3 Me), 0.92 (3 H, *s*, Me), 1.12 (3 H, *s*, Me), 1.21 (3 H, *s*, Me), 2.15 (3 H, *s*, Ac), 3.18 (2 H, *m*, 6′-H), 4.06 (1 H, *t*, $J = 9$ Hz, H-3′ or H-4′), *ca* 4.5 (2′-H, localized by double resonance), 4.98 (1 H, *d*, $J = 8$ Hz, H-1′), 5.39 (1 H, *m*, 12-H), without D_2O signal at 8.90 (*d*, $J = 9$ Hz, NH). EIMS, 10–16eV, *m/z* (rel. int.): 456 $[b + H]^+$ (8), 410 $[b + H–HCO_2H]^+$ (24), 248 $[d]^+$ (100), 207 [retro-Diels–Alder of *b* + H, en-component]$^+$ (71), 203 $[d–CO_2H]^+$ (97). EAMS, 2–4 eV, *m/z*: 456 $[b + H]^-$.



1 R = H
2 R = Ac

Acetyl derivative (**2**). Synthesized from **1** (Ac_2O, pyridine) and cryst.from EtOH; needles, mp 290–295°, $[\alpha]_D^{20}$ +21.3° (pyridine, *c* 0.63). IR ν_{max}^{KBr} cm^{-1}: 1750 (OAc), 1695 (CO_2H), 1655, 1545 (NHAc). EIMS, 10–16 eV *m/z* (rel. int.): 438 $[c - H]^+$ (28), 393 $[c - H - CO_2H]^+$ (38), 330 $[a]^+$ (61), 288 $[a - CH_2CO]^+$ (26), 270 $[a - HOAc]^+$ (26), 248 $[d]^+$ (98), 228 $[a - HOAc - CH_2CO]^+$ (48), 210 $[a - 2HOAc]^+$ (47), 203 $[d - CO_2H]^+$ (100), 168 $[a - 2HOAc - CH_2CO]^+$ (50), 150 $[a - 3HOAc]^+$ (61). EAMS, 2–4eV, *m/z* (rel. int.): 785 $[M]^-$ (6), 725 $[M - HOAc]^-$ (4), 683 $[M - HOAc - CH_2CO]^-$ (12), 455 $[b]^-$ (46), 437 $[b - H_2O]^-$ (31), 167 $[a - 2HOAc - Ac]^-$ (100).

Acid hydrolysis of **1**. Compound **1** refluxed under N_2 with 1 N HCl in EtOH–H_2O (9:1) for 6 hr gave oleanolic acid (identified by mp [6], $[\alpha]_D$ [6], TLC, IR) and 2-amino-2-deoxy-α-D-glucose hydrochloride (identified by $[\alpha]_D$ [7], IR).

β-D-Glucopyranosyl-α-spinasterol. The chromatography of the raw material (see above) over Si gel with $CHCl_3$–MeOH (9:1) gave, in addition to **1**, β-D-glucopyranosyl-α-spinasterol; from MeOH–$CHCl_3$ platelets, yield 0.04% from *P. cubense*, R_f 0.44 (Si gel; $CHCl_3$–MeOH, 4:1); identified by mp [9], $[\alpha]_D$ [9]; EIMS, 10–16 eV *m/z* (rel. int.): 574 $[M]^+$ (45), 531 $[M - Me_2CH]^+$ (in agreement with a 22-double bond [10]) (17), 412 $[aglycone]^+$ (46), 369 $[aglycone - Me_2CH]^+$ (29), 300 (in agreement with a 22-double bond [10] (38), 255 (100), 246 (in agreement with a 7-double bond [11] (46).

REFERENCES

1. Budzikiewicz, H., Wilson, I. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
2. Tori, K., Seo, S., Yoshimura, Y., Nakamura, M., Tomita, Y. and Ishii, H. (1976) *Tetrahedron Letters* 4167.
3. Tori, K., Seo, S., Shimaoka, A. and Tomita, Y. (1974) *Tetrahedron Letters* 4227.
4. Colson, P. and King, R. R. (1976) *Carbohydr. Res.* **47**, 1.
5. Pretsch, E., Clerc, T., Seibl, J. and Simon, W. (1976) *Tabellen zur Strukturaufklärung organischer Verbindungen mit spektroskopischen Methoden*, p. C70. Springer, Berlin.
6. Boiteau, P., Pasich, B. and Ratsimamanga, A. R. (1964) *Les Triterpénoïdes en Physiologie Végétale et Animale*, p. 139. Gauthier-Villars, Paris.
7. Horton, D. (1960) *Advances in Carbohydrate Chemistry*, Vol. 15 (Wolfrom, M. L. and Tipson, R. S., eds.) p. 159. Academic Press, New York.
8. Klyne, W. (1950) *Biochem. J.* **47**, XLI.
9. Karrer, W. (1958) *Konstitution und Vorkommen der organischen Pflanzenstoffe*, p. 860. Birkhäuser, Basel.
10. Wyllie, S. G. and Djerassi, C. (1968) *J. Org. Chem.* **33**, 305.
11. Clark-Lewis, J. W. and Dainis, I. (1967) *Aust. J. Chem.* **20**, 1961.