Phytochemistry, Vol. 22, No. 6, pp. 1469–1471, 1983.
Printed in Great Britain.

0031-9422/83/061469-03$03.00/0

ZIZYNUMMIN, A DAMMARANE SAPONIN FROM *ZIZYPHUS NUMMULARIA*

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(*Revised received* 16 *November* 1982)

Key Word Index—*Zizyphus nummularia*; Rhamnaceae; dammarane saponin; zizynummin.

Abstract—Zizynummin, a new dammarane saponin isolated from dried leaves of *Zizyphus nummularia*, has been assigned the structure β-D-glucopyranosyl-(1 → 2)-6-deoxy-α-L-talopyranosyl-(1 → 3)-α-L-arabinopyranosyl-(1 → 3)-jujubogenin.

INTRODUCTION

Zizyphus nummularia is a wild growing small shrub which is well-known for its medicinal value [1, 2]. Nummularins A–H and K [3–5], manogenin, taxifolin and taxifolin glycoside [6] have been reported from the plant. A new dammarane saponin is reported here from the ethanolic extract of the leaves.

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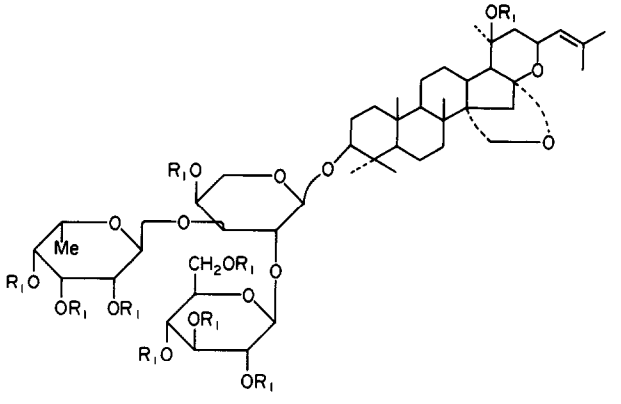
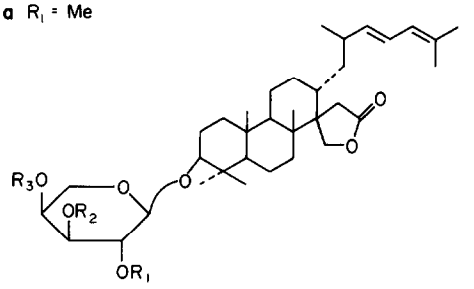
RESULTS AND DISCUSSION

The dried and finely powdered leaves on extraction with ethanol and purification gave a pure saponin which we have named zizynummin (**1**), mp 255–260°; $[\alpha]_D^{20}$ −44.85°. On acid hydrolysis it yielded ebelin lactone, mp 175–180°, $[\alpha]_D^{20}$ −19.5° (IR, UV and EIMS) and L-arabinose, D-glucose, and 6-deoxy-L-talose (co-PC). However, the IR and UV of Zizynummin showed the absence of a lactone ring and conjugated double bond, respectively, therefore, ebelin lactone must be an artefact



1 R_1 = H
1a R_1 = Me



2 R_1 = β-D-Glucose, R_2 = R_3 = H
2a R_1 = 2,3,4,6-Tetra-*O*-methyl β-D-glucose, R_2 = R_3 = Me
3 R_2 = 6-Deoxy-α-L-talose, R_2 = R_3 = H
3a R_2 = 2,3,4-Tri-*O*-methyl-6-deoxy-α-L-talose, R_1 = R_3 = Me

of the real aglycone. Smith–de Mayo degradation [7] (performed twice) of **1** yielded jujubogenin, mp 249–251°, $[\alpha]_D^{20}$ −31.8°, (mmp, co-TLC, EIMS and comparison of ^{1}H NMR) and this compound appears to be the real aglycone of **1**.

The FDMS of **1** showed a cationized cluster ion at *m/z* 935 $[M+Na]^+$ (base peak) and the fragment ion peaks at *m/z* 789 $[(M+Na)-146]^+$, 773 $[(M+Na)-162]^+$ and 627 $[(M+Na)-308]^+$ suggested that **1** was in a pure state and had a branched chain trisaccharide composed of methyl pentosyl–(hexosyl)–pentose in the ratio of 1:1:1 combined with a compound of MW 471 (jujubogenin-H) [8]. In addition, characteristic ions at *m/z* 163 (glucose-OH) and at 147 (6-deoxy-L-talose-OH) were also displayed (Fig. 1) which further proved that two different terminal sugars, namely methyl pentose and hexose, were present in **1** [9]. The ^{1}H NMR signals of three anomeric protons at δ 4.20 (1H, *d*, *J* = 5.5 Hz), 4.26 (1H, *d*, *J* = 7.0 Hz) and 5.28 (1H, *br s*), indicated that the L-arabinose possesses an α-, D-glucose a β- and 6-deoxy-L-talose an α-linkage, which were also supported by the application of Klyne's rule [10].

Methylation of **1** by the modified Hakomori method [11, 12] furnished nona-*O*-methylether, **1a**, which on methanolysis gave a mixture of methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-β-D-glucose, 2,3,4-tri-*O*-methyl-6-deoxy-α-L-talose and 4-mono-*O*-methyl-α-L-arabinose (GC). Hydrolysis of the above sugar mixture gave corresponding methylated sugars (PC). 4-Mono-*O*-methyl-α-L-arabinose was positive to Wallenfels' reagent [13]. The EIMS fragments at *m/z* 219, 187 $[219-MeOH]^+$ and 189, 157 $[189-MeOH]^+$ also confirmed the presence of β-D-glucose and 6-deoxy-α-L-talose as the terminal sugars and also that one of them was attached at C-2 and the other at C-3 of α-L-arabinose which in turn was linked at C-3 with jujubogenin.

Partial hydrolysis of zizynummin afforded two prosapogenins **2** and **3**. The UV spectrum showed that **2** and **3** possess an open chain conjugated triene system [14]. Hydrolysis of **2** furnished ebelin lactone, L-arabinose and D-glucose but **3** afforded 6-deoxy-L-talose instead of D-glucose (co-PC). Hydrolysis of **2** and **3** permethyl ethers, prepared by a modified Hakomori's method, afforded 2,3,4,6-tetra-*O*-methyl-β-D-glucose with 3,4-di-*O*-methyl-α-L-arabinose (also positive to Wallenfels' reagent) and 2,3,4-tri-*O*-methyl-6-deoxy-α-L-talose along with 2,4-di-*O*-methyl-α-L-arabinose, respectively (PC). Moreover, treatment of **3** with sodium periodate liberated free L-arabinose (co-PC).

Therefore, **2** and **3**, formed from the splitting of 6-deoxy-L-talose and D-glucose, respectively, from **1**, possess the structures β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 3)-ebelin lactone and 6-deoxy-α-L-talopyranosyl-(1 → 3)-α-L-arabinopyranosyl-(1 → 3)-ebelin lactone and, hence, zizynummin can be assigned the structure β-D-glucopyranosyl-(1 → 2)-6-deoxy-α-L-talopyranosyl-(1 → 3)-α-L-arabinopyranosyl-(1 → 3)-jujubogenin.

EXPERIMENTAL

Mps are uncorr. PC was carried out on Whatman No. 1 paper using the descending method and aniline hydrogen phthalate as the visualizing agent. The solvent systems used were: (A) $CHCl_3$–MeOH–H_2O (65:25:10); (B) *n*-BuOH–HOAc–H_2O (4:1:5); (C) *n*-BuOH–EtOH–H_2O (5:1:4); (D) C_6H_6–Me_2CO (10:1); (E) C_6H_6–EtOAc (4:1). GC of methylated sugars; column–succinate polyester (10% H.P.), N_2 (30 ml/min), programmed at 175° at 6°/min.

Isolation and purification of zizynummin. Dried and finely powdered leaves of *Zizyphus nummularia*, collected from Meerut (U.P.), were successively extracted with petrol (40–60°), EtOAc and finally with 95% EtOH (× 3, 8 hr). The EtOH extract was dried in *vacuo* and partitioned between H_2O and H_2O satd *n*-BuOH (1:1, × 3). The *n*-BuOH layer was treated with 5% aq. K_2CO_3 soln (twice) and then washed with H_2O. The concd *n*-BuOH extract was subjected to repeated CC (solvent A) to give zizynummin (**1**, 3 g); mp 255–260° (MeOH); $[\alpha]_D^{20}$ −44.85° (MeOH; *c* 1.00); IR ν_{max}^{KBr} cm^{-1}: 3400, 1400; UV λ_{max}^{MeOH} nm: no aborption above 200. FDMS (Fig. 1); ^{1}H NMR (100 MHz, DMSO-d_6): δ 3.09 (1H, *br s*, OH-20) 4.20 (1H, *d*, *J* = 5.5 Hz), 4.26 (1H, *d*, *J* = 7.0 Hz) and 5.28 (1H, *br s*).

Acid hydrolysis of **1**. A soln of **1** (100 mg) in MeOH was hydrolysed by refluxing with 10% aq. HCl (15 ml) for 4 hr, cooled, diluted with H_2O and filtered to afford the ebelin lactone (20 mg). Colourless needles, mp 175–180° (MeOH); $[\alpha]_D^{20}$ −19.5°; IR ν_{max}^{KBr} cm^{-1}: 3500, 1770, 1642, 1600; UV λ_{max}^{MeOH} nm (log ε): 268 (4.58), 278 (4.67) and 288 (4.55); EIMS (probe) 15 eV, *m/z* (rel. int.): 454 $[M]^+$ (0.6), 436 (15), 207 (20), 189 (40), 121 (52), 43 (100). The neutralized (Ag_2CO_3) and concd aq. hydrolysate showed the presence of three sugars only, viz. D-glucose, L-arabinose and 6-deoxy-L-talose (co-PC, authentic samples run in parallel, solvent B, R_f 0.18, 0.21 and 0.44, respectively).

Smith–de Mayo degradation. Compound **1** (500 mg) was oxidized with $NaIO_4$ (1 g in 100 ml at 50% aq. EtOH) at room temp. for 46–60 hr in the dark with continuous stirring. The reaction mixture was refluxed with 5% KOH soln for 4 hr and then extracted with *n*-BuOH (× 3). The final product, obtained after removal of solvent, was submitted to CC (solvent D) to yield pure sapogenin (160 mg). Jujubogenin, colourless needles, mp 249–251° (MeOH); $[\alpha]_D^{20}$ −31.8° (EtOH; *c* 0.8); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH, *br*), 1400; EIMS (probe) 15 eV, *m/z* (rel. int.): 472 $[M]^+$ (0.4), 454 $[M-H_2O]^+$ (19.4), 207 (14.2), 191 (14.7), 189 (16.9), 121 (34.2), 109 (67.1), 97 (100), 43 (41.5); ^{1}H NMR (200 MHz,

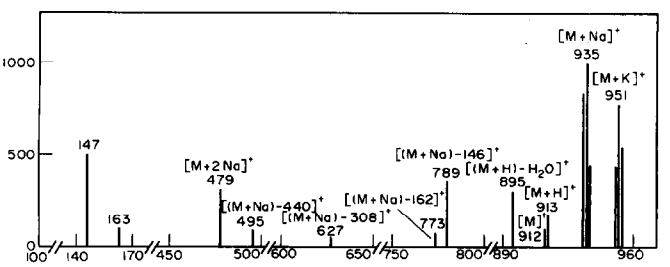


Fig. 1. FDMS of compound **1** between 20 and 45 mA emitter heating current.

C_5D_5N): δ 0.80, 0.95, 1.10, 1.15, 1.33 (3H each, *s*), 1.68 (6H, *br s*), 2.38 (1H, *d*, $J = 8$ Hz), 2.76 (1H, *q*-like), 3.35 (1H, *t*, $J = 8$ Hz), 4.14 (2H, $2 \times d$, $J = 15$ Hz), 4.30 (1H, *br*), 5.35 (1H, *br d*, $J = 7$ Hz) and 5.70 (1H, *br s*, OH).

Permethylation of **1**. Compound **1** (300 mg) was permethylated by the modified Hakomori method to yield the nona-*O*-methyl derivative **1a**, which was purified (CC, solvent E), mp 93.96°; $[\alpha]_D^{20}$ −48.5° ($CHCl_3$; *c* 0.6); IR ν_{max}^{KBr} cm^{-1}: no-OH; EIMS (probe) 15 eV, *m/z* (rel. int.): 833 [M − tri-*O*-methyl-6-deoxy-L-talose + H]$^+$ (0.3), 803 [M − tetra-*O*-methyl-glucose + H]$^+$ (0.4), 454 (4.4), 219 (10.9), 189 (100), 187 (92.5), 157 (70.8), 155 (13.7), 101 (100), 88 (100) and 45 (25.6). (Found: C, 64.28; H, 9.10. $C_{56}H_{94}O_{17}$ requires: C, 64.71; H, 9.12%.)

Methanolysis of **1a**. Compound **1a** (100 mg) in 1 N HCl–MeOH (15 ml) was refluxed (3 hr), neutralized (Ag_2CO_3), diluted, filtered and the concd filtrate was subjected to GC, R_t (min): 2,3,4,6-tetra-*O*-methyl-β-D-glucose (2.02); 2,3,4-tri-*O*-methyl-6-deoxy-α-L-talose (2.87); 4-mono-*O*-methyl-α-L-arabinose (8.59). Hydrolysis of a portion of the above sugar mixture with 10% HCl showed the presence of the following sugars on PC (solvent C); 4-mono-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-6-deoxy-L-talose and 2,3,4,6-tetra-*O*-methyl-D-glucose (R_G: 0.38, 0.88 and 1.0, respectively). On spraying with Wallenfels' reagent the spot corresponding to R_G value 0.38 gave an intense pink colour.

Partial hydrolysis. A soln of zizynummin (1 g) in 5% aq. HCl–MeOH (1:1, 50 ml) was heated under reflux on a steam bath for 45 min. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness *in vacuo* and the residue was separated by CC (solvent A) to give ebelin lactone (50 mg) and two prosapogenins. Compound **2** (150 mg); mp 260–265° (MeOH); UV λ_{max}^{MeOH} nm (log ε): 266 (4.58), 276 (4.67) and 288 (4.55). Compound **3** (175 mg) mp 255–260° (MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400, 1765, 1640, 1600; UV λ_{max}^{MeOH} nm (log ε): 267 (4.58), 277 (4.67) nd 288 (4.55). Compounds **2** and **3** (25 mg) were separately hydrolysed and worked-up in the usual way to yield ebelin lactone (mmp, co-TLC and IR) and neutral concd aq. hydrolysates of **2** and **3** showed D-glucose (R_f 0.18) and L-arabinose (R_f 0.21); L-arabinose and 6-deoxy-L-talose (R_f 0.44), respectively (co-PC with authentic samples, solvent B).

Permethylation and methanolysis of **2** *and* **3**. Portions (75 mg) of both **2** and **3** were separately permethylated by the modified Hakomori method to give their per-*O*-methyl derivatives **2a** and **3a**, respectively. Compound **2a**: mp 78–80°; IR ν_{max}^{KBr} cm^{-1}: no OH; EIMS (probe) 15 eV, *m/z* (rel. int.): 832 [M]$^+$ (0.4), 597 (0.3), 219 (20), 187 (80), 88 (100). Compound **3a** mp 87–90°; IR ν_{max}^{KBr} cm^{-1}: no OH; EIMS (probe) 15 eV, *m/z* (rel. int.): 802 [M]$^+$ (0.3), 597 (0.6), 189 (100) and 157 (22). Compounds **2a** and **3a** were methanolysed followed by hydrolysis and work-up in the usual way. The methyl sugars from **2a** were identified (PC, solvent C) as 3,4-di-*O*-methyl-L-arabinose (R_G 0.65), positive to Wallenfels' reagent, and 2,3,4,6-tetra-*O*-methyl-D-glucose (R_G 1.0). Compound **3a** gave 2,4-di-*O*-methyl-L-arabinose (R_G 0.65) and 2,3,4-tri-*O*-methyl-6-deoxy-L-talose (R_G 0.88).

Periodate oxidation of **3**. Compound **3** (25 mg) in H_2O (10 ml) was mixed with $NaIO_4$ (250 mg) and the soln kept in the dark for 48 hr. Ethylene glycol (1 ml) was added to decompose excess $NaIO_4$ and the soln was hydrolysed with 10% MeOH–HCl (45 min). Its filtrate was neutralized, concd and examined by PC (solvent B) to detect L-arabinose (R_f 0.21) only.

Acknowledgements—We are grateful to Professor K. Yamasaki, Hiroshima University for FDMS and a sample of 6-deoxy-L-talose; to Professor S. Shibata, Japan, for a sample of jujubogenin; to Dr. J. A. Salazar, Spain, for EIMS; to Professor K. Hiller, East Germany, for ^{1}H NMR; and to Dr. (Miss) V. Dabral, NCL, India, for GC. Thanks are also due to Dr. O. P. Sharma, H. P. Agriculture University, Palampur, India for the identification of plant material. R. K. thanks the UGC, New Delhi, for a teacher fellowship.

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