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TRITERPENOID SAPONINS FROM THE BARK OF *ZIZYPHUS JOAZEIRO*

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Abstract—Three new triterpenoid saponins were isolated from the barks of *Zizyphus joazeiro* and characterized as jujubogenin 3-*O*-α-L-arabinofuranosyl-(1 → 2)-[β-D-glucopyranosyl(1 → 3)]-α-L-arabinopyranoside, its 4‴-*O*-sulphate and 3″,4‴-di-*O*-sulphate, respectively FAB-MS was useful in providing information on the molecular weight of the complex oligoglycoside sulphates

INTRODUCTION

The bark of *Zizyphus joazeiro* Mart has been known as a folk medicine in Brazil, for example as a detergent [1] Concerning their constituents a cyclopeptide alkaloid (amphibine-D) [2], betulinic acid and an uncharacterized saponin, which upon acid hydrolysis gave ebelin lactone [3], were reported This paper describes the isolation and structure determination of three new triterpenoid saponins (jujubogenin 3-*O*-glycoside and its sulphates) from the bark of *Z joazeiro*

RESULTS AND DISCUSSIONS

The methanol extractives of the bark were separated successively by normal-phase, sephadex and reversed-phase (RP) CC, and the fractions showing a single spot on normal- and RP-TLC were crystallized and precipitated to give three compounds **1**, **2** and **3**

These compounds were hydrolysed with acid to yield glucose and arabinose together with ebelin lactone (**4**) which is obtained from jujubogenin glycoside by acid hydrolysis [4] The IR spectra of compounds **1**–**3** showed no absorption due to a lactone ring, and their ^{13}C NMR spectra (Table 1) gave the signals assignable to the aglycone carbons which were in good agreement with those of jujubogenin 3-*O*-glycoside [5] The above data indicate that **1**–**3** are 3-*O*-glycosides of jujubogenin possessing the glucose and arabinose units in the sugar moieties

Compound **1** showed in the FAB-MS the molecular ion peak as a cationized cluster ion ($[M(C_{46}H_{74}O_{17})+K]^+$) at *m/z* 937 and in the ^{13}C NMR spectrum three anomeric carbon signals appeared at δ104 9, 105 7 and 110.3 Therefore, compound **1** is regarded to consist of one mole each of jujubogenin and glucose and two moles of arabinose Compound **2** as well as **3** gave **1** on solvolysis using a dioxane–pyridine mixture [6], and showed absorption due to a sulphate group in the IR spectrum. Therefore, both **2** and **3** must be sulphates of **1** In the FAB-MS of **2** and **3** the molecular ion peaks were observed at *m/z* 1055 and 1173, $[M(C_{46}H_{73}O_{17}\ SO_3K)+K]^+$ and $[M(C_{46}H_{72}O_{17}\ 2SO_3K)+K]^+$), respectively, and their elemental analytical data indicated the existence of one and two sulphur atoms, respectively Therefore, compound **2** is regarded as a monosulphate and **3** as a disulphate of **1**

The structure of the oligosaccharide moiety of **1** and the site of linkage of the sulphate groups in **2** and **3** were determined as follows Permethylation of **1** by the Hakomori method [7] followed by methanolysis of the product (**5**) provided **4** and three methylated monosaccharides, which were identified as methyl pyranosides of 4-*O*-methyl-arabinose (S-1), 2,3,4,6-tetra-*O*-methylglycose (S-2) and methyl furanoside of 2,3,5-tri-*O*-methylarabinose (S-3) Thus, the arabinofuranose and glucopyranose units in the sugar moiety of **1** are combined, respectively, with the 2- and 3- or 3- and 2-hydroxy groups of arabinopyranose, and hence, if arabinose and glucose are assumed to be the most commonly found L- and D-series, **1** is jujubogenin 3-*O*-L-arabinofuranosyl-(1 → 2 or 1 → 3)-[D-glucopyranosyl (1 → 3 or 1 → 2)]-L-arabinopyranoside The configurations of arabinopyranosyl, glucopyranosyl and arabinofuranosyl units were considered, respectively, to be α, β and α by their anomeric proton signals (*d*, *J* = 5 Hz, *d*, *J* = 7 Hz, and singlet) [5, 8] in the ^{1}H NMR spectrum of **5**

The location of the sulphate moiety in **2** was determined by chemical and ^{13}C NMR spectral data Methanolysis of permethylate (**6**) of **2** afforded S-1, S-3 and methyl pyranoside of 2,3,6-tri-*O*-methyl-glucose (S-4) along with **4** indicating that the sulphate group is combined with the 4-hydroxy group of glucose The signal due to C-4‴ of glucose in **2** shifted to lower field compared with that in **1** on esterification [9, 10] (Table 1) The position of the sulphate groups in **3** were suggested by comparison of the ^{13}C NMR spectra of **1** and **3** (Table 1) Namely, the signals due to C-3″ of arabinofuranose and C-4‴ of glucose in **3** shifted to lower field on esterification indicating that the

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Table 1 ^{13}C NMR spectral data (C_5D_5N) for compounds 1, 2 and 3

C	1	2	3	saponin C$_2^*$	C†	1	2	3
1	38 8	38 8	38 8	39 0	1′	104 9	104 6	104 4
2	26 8	26 7	26 8	26 7	2′	77 0	76 7	77 6
3	88 7	88 7	88 7	88 1	3′	83 8	83 6	83 1
4	39 8	39 8	39 8	39 7	4′	68 5	68 6	68 7
5	56 2	56 2	56 2	56 3	5′	65 9	65 9	65 8
6	18 4	18 3	18 4	18 3				
7	36 0	36 2	36 0	36 0	1″	110 3	110 0	109 9
8	37 5	37 5	37 5	37 3	2″	83 5	83 6	82 0b
9	53 0	53 0	53 0	53 0	3″	78 4	77 7	82 5b
10	37 2	37 2	37 1	37 3	4″	84 9	84 8	83 1b
11	21 8	21 8	21 8	21 8	5″	62 1a	61 8	61 7c
12	28 5	28 5	28 5	28 6				
13	37 2	37 2	37 1	37 5	1‴	105 7	105 6	105 2
14	53 7	53 7	53 7	53 7	2‴	75 2	74 8	75 1
15	36 9	37 2	37 1	37 1	3‴	78 0	76 0	76 2
16	110 6	110 6	110 6	110 6	4‴	71 4	76 0	76 2
17	53 9	53 9	53 9	53 9	5‴	78 0	76 0	76 2
18	18 4	18 3	18 4	18 3	6‴	62 5a	61 8	62 0c
19	16 4	16 4	16 4	16 4				
20	68 5	68 4	68 4	68 5				
21	30 0	30 0	30 0	30 0				
22	45 4	45 5	45 4	45 5				
23	68 5	68 6	68 7	68 5				
24	127 1	127 0	126 9	127 0				
25	134 1	134 1	134 3	134 2				
26	25 6	25 6	25 6	25 5				
27	18 9	18 8	18 8	18 9				
28	27 8	27 7	27 7	28 0				
29	16 6	16 5	16 4	16 8				
30	65 9	65 9	65 8	65 8				

a, b, c Assignments may be reversed in each vertical column

*Jujubogenin 3-*O*-glycoside obtained from *Hovenia dulcis* [5]

†By taking the glycosidation shift [11, 12] into account and comparing with the spectra of reference compounds, the signals due to sugar moieties of each glycoside were assigned

3- and 4-hydroxy groups of arabinofuranose and glucose, respectively, are sulphated

Hydrolysis of **2** with a crude hesperidinase afforded a prosapogenin **7** Examination of the acid hydrolysate and the FAB-MS and ^{13}C NMR spectral data of **7** indicated that **7** was a diglycoside where only arabinofuranose of **2** was removed Methanolysis of the permethylate (**8**) of **7** afforded S-4 and the methyl pyranoside of 2,4-di-*O*-methyl-arabinose (S-5)

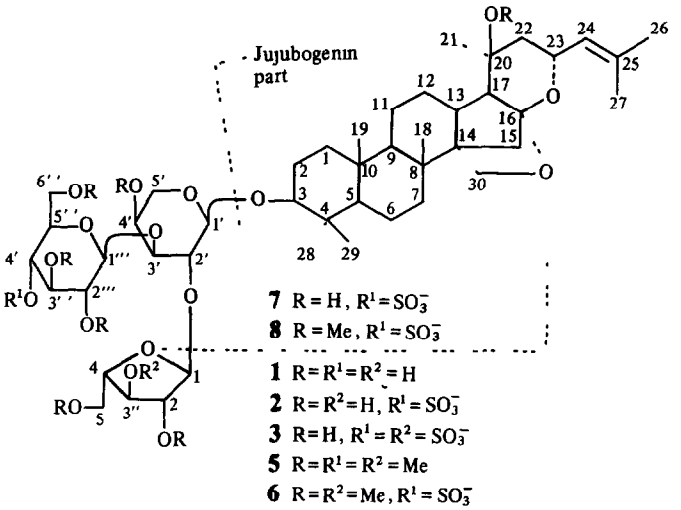
Therefore, **7** is jujubogenin 3-*O*-*β*-D-glucopyranosyl-(1 → 3)-*α*-L-arabinopyranoside 4‴-*O*-sulphate, and hence, the arabinofuranose and glucose units are attached, respectively, to the 2- and 3-hydroxy groups of arabinopyranose

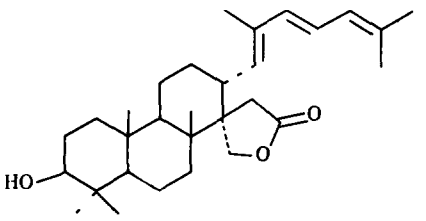
Consequently, **1** is jujubogenin 3-*O*-*α*-L-arabinofuranosyl-(1 → 2)-[*β*-D-glucopyranosyl(1 → 3)]-*α*-L-arabinopyranoside, and **2** and **3** are 4‴-*O*-sulphate and 3″,4‴-di-*O*-sulphate of **1** Several other jujubogenin 3-*O*- and 3,20-*O*-bis-glycosides have been reported [5, 13–17]

EXPERIMENTAL

All mps were uncorr Optical rotations were measured at 20–28° using 1 dm cell ^{1}H NMR spectra were taken at 100 MHz in $CDCl_3$ soln unless otherwise specified using TMS as internal standard ^{13}C NMR spectra were recorded at 25 05 MHz in C_5D_5N (TMS as internal standard) employing the FT mode The EI- and FAB-MS were produced on double focusing mass spectrometers The former was taken with an accelerating potential of 3 kV and an ionizing potential of 30 eV, and the latter at 2 kV for the ion source and 6 kV for an argon beam source and the spectra were obtained from glycerol solutions using KI as additive Conditions of GLC [FID mode, glass column (1 2 m × 4 mm) packed with 10% 1,4-butanediol succinate on shimalite W(60–80 mesh)] (a) column temp 160°, N_2 carrier gas (0 6 kg/cm^2), (b) 167°, 0 8 kg/cm^2 Solvent systems of TLC (silica gel, C-18 (reversed-phase) and Avicel) (a) $CHCl_3$–MeOH–H_2O (25 17 3), (b) *n*-hexane–EtOAc (2 1), (c) EtOAc–MeOH (50 1), (d) 60% MeOH, (e) upper layer of *n*-BuOH–AcOH–H_2O (4 1 5)

Isolation Zizyphus joazeiro was collected in the dry region of Brazil's northeast A herbarium specimen of the plant is on file in the Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University The bark (10 kg) was extracted with MeOH and the MeOH evaporated *in vacuo* to leave the MeOH extract (215 g) The extract was chromatographed on silica gel (eluant $CHCl_3$–MeOH–H_2O, 65 35 10) to give three crude saponin fractions, Fr 1 (500 mg), Fr 2 (7 5 g) and





4

Fr 3 (3 5 g) Each fraction was passed through a Sephadex LH-20 column (eluant, MeOH) to give saponin fractions Fr' 1 (150 mg), Fr' 2 (1 78 g) and Fr' 3 (1 0 g) showing a single spot on TLC (silica gel) Fr' 1, 2 and 3 showed two spots (major and minor) on TLC (C-18) and were separated by reversed-phase (C-18) CC (eluant 70%, 55% and 40% MeOH) to give each major component The major components were crystallized and precipitated to give **1** (33 mg), **2** (140 mg) and **3** (60 mg) [R_f 0 52, 0 41 and 0 28 silica gel (a), R_f 0 09, 0 47 and 0 74 C-18 (d)]

Compound **1** Colorless needles (from dil MeOH), mp 250–252° (decomp), $[\alpha]_D$ −39 9° (MeOH, *c* 1 1) IR ν_{max}^{KBr} cm^{-1} 3400 (OH) FAB-MS *m/z* 937 $[M(C_{46}H_{74}O_{17})+K]^+$ Compound **1** was refluxed with 2 N H_2SO_4 in 50% EtOH for 2 hr and then diluted with H_2O The precipitates were collected by filtration, dried, and subjected to preparative TLC ($CHCl_3$–Me_2CO, 20 1) to afford **4** as an amorphous powder, mp 73–75° IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3500 (OH), 1770 (γ-lactone) EI-MS *m/z* 454 $[M]^+$ ^1H NMR δ0 78, 0 86, 0 99, 1 04 (3H each, *S*, 4 × Me), 1 78, 1 80, 1 81 (3H each, *s*, 3 × Me) Compound **4** was identified as ebelin lactone by direct comparison (IR and ^1H NMR) The filtrate was neutralized with $Ba(OH)_2$ soln, filtered and the filtrate was concd and examined by TLC [Avicel (e)], and arabinose and glucose were detected

Compound **2** Amorphous powder (from MeOH), mp 208–214° (decomp), $[\alpha]_D$ −27 5° (MeOH, *c* 1 7) IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1230, 825 (sulphate) FAB-MS *m/z* 1055 $[M(C_{46}H_{73}O_{17}\ SO_3K)+K]^+$ (Found S, 3 13 $C_{46}H_{73}O_{17}\ SO_3^-$ requires S, 3 28%) On hydrolysis with acid as described for **1** compound **2** gave **4** besides arabinose and glucose [TLC Avicel (e)]

Compound **3** Amorphous powder (from H_2O–*n*-BuOH), mp 184–187° (decomp), $[\alpha]_D$ −12 6° (MeOH, *c* 1 7) IR ν_{max}^{KBr} cm^{-1} 3450, 3200 (OH), 1230, 820 (sulphate) FAB-MS *m/z* 1173 $[M(C_{46}H_{72}O_{17}\ 2SO_3K)+K]^+$ (Found S, 5 39 $C_{46}H_{72}O_{17}\ 2SO_3^-$ requires S, 6 07%) Compound **3** afforded **4**, arabinose and glucose on acid hydrolysis

Solvolysis of **2** *and* **3** The soln of **2** (3 mg) in pyridine (1 ml) was heated under reflux for 10 min, then dioxane (4 ml) was added, and the mixture was refluxed for a further 25 min The reaction mixture was diluted with H_2O, extracted with *n*-BuOH and the *n*-BuOH layer was washed and evapd The residue (solvolysate) showing a single spot on TLC was identified with **1** by normal- and reversed-phase TLC [silica gel (a) and C-18 (d)] Compound **3** was solvolysed as **2** and yielded **1**

Methylation of **1** *by the Hakomori method* Compound **1** (20 mg) was treated with NaH (100 mg) and MeI (1 ml) in DMSO (5 ml) The reaction mixture was diluted with H_2O, extracted with $CHCl_3$ and the $CHCl_3$ layer was washed, dried and evapd The residue was chromatographed on silica gel *n*-hexane–Me_2CO (3 1) to give **5** (15 mg) as an amorphous powder IR $\nu_{max}^{CCl_4}$ cm^{-1} no OH ^1H NMR δ0 82 (6H, *s*, 2 × Me), 0 97 (3H, *s*, Me), 1 06 (6H, *s*, 2 × Me), 1 70 (6H, *s*, 2 × Me), 3 16, 3 40, 3 43, 3 50, 3 57, 3 59 (3H each, *s*, 6 × OMe), 3 38 (9H, *s*, 3 × OMe), 4 40 (1H, *d*, *J* = 5 Hz, anomeric H of arabinopyranose), 4 49 (1H, *d*, *J* = 7 Hz, anomeric H of glucopyranose), 5 36 (1H, *s*, anomeric H of arabinofuranose), 5 22 (1H, broad *d*, *J* = 8 Hz, H-24)

Methanolysis of **5** Compound **5** was heated with 10% HCl in MeOH for 2 hr, the mixture was treated with Ag_2CO_3 and filtered The filtrate was evapd and the residue (methanolysate) was examined by TLC [silica gel (b) and (c)] and GLC [condition (a)], and **4** and three methylated sugars were detected and they were identified as S-1, S-2 and S-3 by comparison with the authentic samples

Permethylate **6** *of* **2** (*Hakomori method*) Compound **2** was methylated by the Hakomori method, treated as in **1**, and the crude methylated product was chromatographed on silica gel (*n*-hexane–Me_2CO, 2 1) to give **6** as an amorphous powder

IR $\nu_{max}^{CCl_4}$ cm^{-1} no OH ^{1}H NMR ($CDCl_3 + C_5D_5N$) δ0 80, 0 88 (3H each, *s*, 2 × Me), 1 05 (9H, *s*, 3 × Me), 1 68 (6H, *s*, 2 × Me), 3 14, 3 32, 3 34, 3 39, 3 43, 3 44 (3H each, *s*, 6 × OMe), 3 62 (6H, *s*, 2 × OMe), 4 48 (1H, *d*, J = 5 Hz, anomeric H of arabinopyranose), 4 65 (1H, *d*, J = 7 Hz, anomeric H of glucopyranose), 5 55 (1H, *s*, anomeric H of arabinofuranose), 5 28 (1H, *br d*, J = 8 Hz, H-24)

Methanolysis of **6** Compound **6** was methanolysed and worked up in the same manner as in **5** The methanolysate was examined by TLC [silica gel (b) and (c)] and GLC [condition (b)], and **4**, S-1, S-3 and S-4 were detected

Partial hydrolysis of **2** *with a crude hesperidinase* Compound **2** (100 mg) in H_2O was incubated with a crude hesperidinase at 39° for 4 days, and the product was extracted with *n*-BuOH The extracts showing a major spot (R_f 0 36) on TLC (silica gel, $CHCl_3$–MeOH–H_2O, 7 3 0 1) were evapd and chromatographed on silica gel ($CHCl_3$–MeOH–H_2O, 7 3 0 1) to give **7** as an amorphous powder (30 mg) (from MeOH), mp 232–235° (decomp) IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1225, 830 (sulphate) FAB-MS *m/z* 923 [M($C_{41}H_{65}O_{13}SO_3K$) + K]$^+$ ^{13}C NMR δ105 7 (anomeric C), 107 4 (anomeric C) Compound **7** gave **4**, arabinose and glucose on acid hydrolysis as before

Preparation of **8** *and its methanolysis* Compound **7** was methylated in the same manner as in **1** to give **8** as an amorphous powder IR $\nu_{max}^{CCl_4}$ cm^{-1} no OH ^{1}H NMR δ0 82 (6H, *s*, 2 × Me), 1 00 (3H, *s*, Me), 1 06 (6H, *s*, 2 × Me), 1 67, 1 70 (3H each, *s*, 2 × Me), 3 15, 3 36, 3 44, 3 58 (3H each, *s*, 4 × OMe), 3 62 (6H, *s*, 2 × OMe), 4 17 (1 H, *d*, J = 7 Hz, anomeric H of glucopyranose), 4 52 (1 H, *m*, $W_{1/2}$ = 9 Hz, anomeric H of arabinopyranose), 5 22 (1 H, *br d*, J = 8 Hz, H-24) Compound **8** was methanolysed as before to give **4**, S-4 and S-5 [TLC silica gel (b) and (c), GLC condition (b)]

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