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PSEUDOJUJUBOGENIN, A NEW SAPOGENIN FROM *BACOPA MONNIERA*

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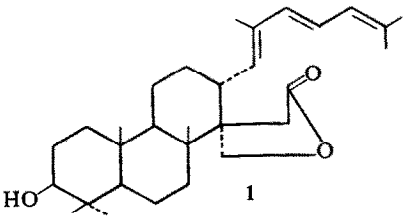
Key Word Index—*Bacopa monniera*; Scrophulariaceae; bacogenin A_1; bacoside A; jujubogenin; ebelin lactone; pseudojujubogenin.

Abstract—Acid hydrolysis of bacoside A, a saponin of *Bacopa monniera*, afforded ebelin lactone and bacogenin-A_1, while Smith-de Mayo degradation of bacoside A yielded jujubogenin and pseudojujubogenin as sapogenins. The structure of pseudojujubogenin except the absolute configurations at C-20 and C-22 was established by chemical and spectroscopic investigations. Bacoside A was separated by droplet counter-current chromatography into two fractions, A_1 and A_2, which, however, are mixtures, since they both afforded two sapogenins, in different ratios by Smith-de Mayo degradation.

From the Indian medicinal plant *Bacopa monniera* Wettst. Rastogi *et al.* [1] isolated a saponin, bacoside A. Acid hydrolysis of bacoside A yielded ebelin lactone (**1**) and bacogenin A_1 (**2**) [2] besides D-glucose and L-arabinose [3]. The structure of bacogenin A_1 (**2**) was established by the X-ray crystallography of its dibromoacetate (**3**) [4]. However, both sapogenins **1** and **2** must be artifacts produced by the action of acid, since bacoside A showed no evidence of possessing a lactone and a conjugated double bond in the IR and UV spectra, as in the case of jujuboside B of *Zizyphus jujuba* Mill. and hovenoside G of *Hovenia dulcis* Thunb., both of which also produced ebelin lactone (**1**) on acid hydrolysis [5]. On treatment with periodate and alkali, bacoside A afforded a new sapogenin, $C_{30}H_{48}O_4$, mp 273–276°, named pseudojujubogenin (**6**), along with jujubogenin (**4**) as the main products. Previously, the structure of jujubogenin including the absolute configuration at C-20 and C-23 was established [5, 6] as **4** by the chemical evidence as well as by the X-ray crystallography of its monobromobenzoate (**5**). Jujubogenin is the genuine sapogenin of jujuboside B and hovenoside G. By the action of sulphuric acid, pseudojujubogenin (**6**) was converted into bacogenin A_1 (**2**), without producing ebelin lactone (**1**). The IR and UV spectra of pseudojujubogenin (**6**) indicate the absence of carbonyl and conjugated double bond system in the molecule as in the parent saponin. From the above results and the similarity in the PMR and IR spectra of pseudojujubogenin with those of jujubogenin (**4**), it is suggested that pseudojujubogenin is also a dammarane-type triterpene. On acetylation **6** afforded a monoacetate (**7**), $C_{32}H_{50}O_5$, mp 255–257°, in which one tertiary hydroxyl (IR : 3460 cm^{-1}) remains free. The PMR signal of a proton attached to the carbon bearing a hydroxyl which appears at δ 3.20 (q, J = 10 and 5 Hz) in **6** is shifted to δ 4.44 (q, J = 8 and 6 Hz) in its monoacetate (**7**), and a PMR signal of acetoxyl protons appears at δ 2.03 (s),



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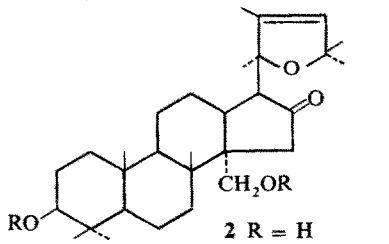
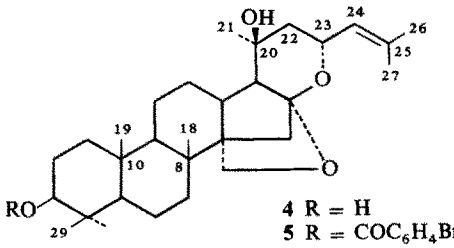
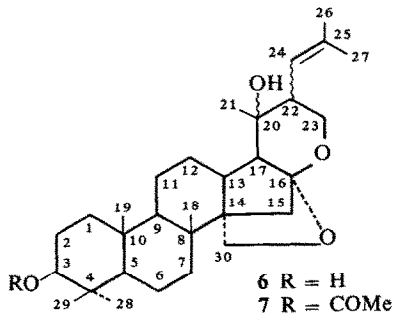


2 R = H
3 R = $COCH_2Br$



4 R = H
5 R = COC_6H_4Br



6 R = H
7 R = COMe

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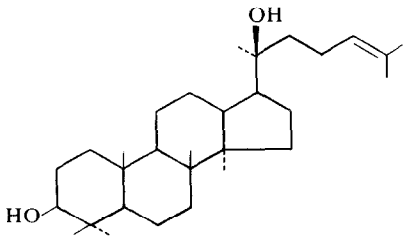

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without any significant change in other PMR signals. Therefore, a secondary hydroxyl of **6** must be located at C-3 -β as in **2** and **4**, while there is a tertiary hydroxyl at at C-20. Thus two out of four oxygen functions of **6** have been assigned to two hydroxyls, and the remaining two oxygens could be involved in ethers of a ketal group. The signal at δ 3.98 (*br s*) in **7** is assigned to a methylene attached to a primary oxygen function, while δ 3.57 (*q*, J = 12 and 4 Hz) and δ 4.11 (*q*, J = 12 and 4 Hz) to proton attached to a carbon bearing an oxygen function. The latter is the only difference between the signals of **7** and the acetate of **4**. The signal at δ 5.29 (*br d*, J = 11 Hz) in **7** is assigned to a vinyl proton, which is converted into a broad singlet when the proton at δ 2.34 (*m*) is irradiated. Furthermore, the signals at δ 3.57 and δ 4.11 are converted into doublets (J = 12 Hz) by the irradiation at δ 2.34. No remarkable change of PMR spectrum is shown except the signal at δ 2.34, when the proton at δ 5.29 is irradiated. Moreover, the signals at δ 3.57 and 4.11 are coupled each other. These results suggested the presence of a side chain C—CH(CH_2O—)CH=CMe_2 in the structure of **7** and, consequently in **6**. From the above PMR data referring to the structure of **2** and **4**, pseudojujubogenin would be represented by (**6**).

Furthermore, the ^{13}C-NMR of **6** in d_5-pyridine was compared with those given by **4** and dammaranediol-II (**8**) [7]. The signals at δ 123.9 and 132.6 of 6 were assigned to the vinylic carbons at 24 and 25 positions, and the signals at δ 110 of **6** as a ketal carbon at 16. Then the signals of **6** at δ 77.8, 71.6, 66.0 and 65.8 were assigned to four carbons bearing oxygen function at the 3, 20, 23 and 30 position, respectively. In the off-resonance decoupled spectra, 4 gave a triplet signal at δ 45.3 for C-22 and a doublet at δ 68.5 for C-23, whereas **6** gave a doublet at δ 46.0 and a triplet at δ 66.0, which must be assigned to C-22 and C-23, respectively. Hence it has been proved that an isobutenyl side chain is located at C-22 of 6. The absolute configurations at C-20 and C-22 of 6 remain unsettled. The conversion of **6** into bacogenin-A_1 (2) by mineral acids could be explained as in Scheme 1.

Meanwhile, bacoside A was separated into fractions A_1 and A_2 by droplet counter-current chromatography (DCC) [8] using $CHCl_3$–MeOH–H_2O (5.6:4). On treatment with sulphuric acid, A_1 and A_2 also afforded ebelin lactone (**1**) and bacogenin-A_1 (**2**), in the ratios 1:2 and 2:1 from A_1 and A_2 respectively. The fractions A_1 and A_2 gave no absorptions due to carbonyl and conjugated double bond systems in the IR and UV spectra, while they afforded (**4**) and (**6**) on treatment with periodate though the ratio of products were different between A_1 and A_2. Consequently, A_1 and A_2 are still mixtures although they gave well separated peaks in DC-chromatogram, and one of the components yields (**4**) and the other (**6**) on Smith-de Mayo degradation. The saponin component whose genuine sapogenin is pseudojujubogenin (**6**) would yield bacogenin A_1 on acid hydrolysis.

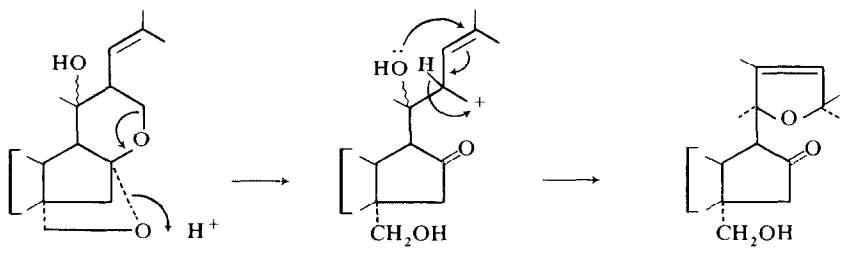
After completion of this work, the authors have noted the appearance of a paper on bacogenin A_3 [9] the structure of which corresponds to anhydropseudojujubogenin. The structural formula of (**6**) is also referred to. Bacogenin A_3, one of the acid hydrolysates of bacoside A, is obviously an artifact, while pseudojujubogenin is regarded as a genuine sapogenin.

EXPERIMENTAL

Mps are uncorr. The IR spectra were measured in KBr pellets. The PMR spectra were recorded at 100 MHz and the ^{13}C-NMR spectra at 15 or 25 MHz.

Periodate oxidation of bacoside A. Bacoside A (2 g) was oxidised with $NaIO_4$ (4 g in 200 ml 50% aq MeOH) at room temp. for 48 hr, and a few drops of ethylene glycol were added to destroy excess $NaIO_4$. The reaction mixture was extracted with BuOH, and the butanolic layer was refluxed with 5% KOH in 20% aq. EtOH for 3 hr and then extracted with BuOH. The above procedure was repeated twice. The final products were chromatographed on Si gel eluting with C_6H_6–Me_2CO (10:1) to give two main sapogenins. The later eluate gave colourless needles, mp 250–252° from MeOH (yield 80 mg) which was identified as jujubogenin (**4**) by IR, PMR, MS, TLC and a mmp. The earlier eluate afforded colourless needles, mp 273–276° from MeOH (yield: 101 mg), which is a new sapogenin, named pseudojujubogenin (**6**).

Pseudojujubogenin (**6**). Colourless needles, mp 273–276° from MeOH. ν_{max}^{KBr}. 3520, 3400 (OH), 1469, 1285, 1073, 1007 cm^{-1}. λ_{max}^{EtOH}. no absorption above 210 nm. ORD. (−)-plain curve (EtOH). δ in $CDCl_3$ 0.77, 0.83, 0.96, 1.07 (3H each, *s*, tert.-CH_3 × 4), 1.10 (3H, *s*, HO—C—CH_3), 1.65, 1.73 (3H, each, *br s*, CH_3—C—CH_3), 1.91 (1H, *d*, J = 8 Hz) 2.35 (2H, *m*), 3.20 (1H, *q*, J = 10 and 5 Hz. >C(OH)H), 3.58 (1H, *q*, J = 12 and 4 Hz), 4.00 (2H, *br s*, —O—CH_2—C≤), 4.14 (1H, *q*, J = 12 and 4 Hz), 5.25 (1H, *br d*, J = 12 Hz. >CH—CH=). *m/e*. 454 (M-H_2O), 436 (M-2H_2O), 424, 421, 418, 409, 391 (bp), 390 (Observed: 454.3569 Calcd. for $C_{30}H_{46}O_3$ (M-H_2O): 454.3445)



6 2

Scheme 1.

Table 1. ^{13}C-NMR spectra of jujubogenin (**4**) and pseudojujubogenin (**6**) (δ ppm in d_5-pyridine)

Compound	4	6		4	6		4	6
C-1	38.8	38.8	C-11	21.7	21.6	C-21	30.0	27.0
C-2	28.1	28.0	C-12	28.6	28.5	C-22	45.3	46.0
C-3	77.8	77.8	C-13	37.0	36.9	C-23	68.5	66.0‡
C-4	39.5	39.4	C-14	53.8	53.3	C-24	126.9	123.9
C-5	55.9	55.9	C-15	36.1(?)	29.8	C-25	134.0	132.6
C-6	18.3	18.4	C-16	110.4	110.0	C-26	25.5	26.0
C-7	36.1	36.0	C-17	53.9	51.1	C-27	18.9†	18.7†
C-8	37.5*	37.4*	C-18	18.5†	18.4†	C-28	28.6	28.5
C-9	53.0	52.9	C-19	16.3†	16.3†	C-29	16.3†	16.3†
C-10	36.7*	36.8*(?)	C-20	68.4	71.6	C-30	65 8	65.8‡

*, †, ‡: Values given by each compound may be reversed

On acid hydrolysis, (**6**) yielded (**2**) along with some other products, but (**1**) could not obviously be detected.

Pseudojujubogenin monoacetate (*7*). (**6**) (20 mg) was acetylated with Ac_2O (1 ml) and Py (2 ml) to obtain pseudojujubogenin monoacetate (**7**) (13 mg) as colourless needles, mp 255–257° from MeOH. ν_{max}^{KBr} : 3460 (OH), 1734, 1244 (OAc) cm^{-1}. λ_{max}^{EtOH} : end absorption only. δ in $CDCl_3$ 0.85 (9H, *s*, tert.-CH_3 × 3), 1.06 (3H, *s*, tert.-CH_3), 1.09 (3H, *s*, $\geq$C(OH)—CH_3), 1.64, 1.73 (3H, each, *br s*, CH_3—C—CH_3), 1.89 (1H, *d*, J = 9 Hz), 2.03 (3H, *s*, —OCOCH_3), 2.34 (2H, *m*), 3.57 (1H, *q*, J = 12 and 4 Hz), 3.98 (2H, *br s*, $\geq$C—CH_2—O—), 4.11 (1H, *q*, J = 12 and 4 Hz), 4.44 (1H, *q*-like, J = 8 and 6 Hz, $\geq$C(OAc)—H, 5.29 (1H, *br d*, J= 11 Hz, $\geq$CH—CH=. *m/e*. 496 (M-H_2O), 436, 391, 372, 371. Anal.; found. C, 74.40; H, 9.81. Calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. (Observed : 496.3445. Calcd. for $C_{32}H_{48}O_4$ (M-H_2O), 496.3550).

Separation of fractions A_2 and A_2 from bacoside A. Bacoside A (1.6 g) was subjected to DCC using $CHCl_3$–MeOH–H_2O (5:6.4) to separate into fraction A_1 (yield. 710 mg) and A_2 (yield. 370 mg). The fractions A_1 and A_2 afforded also on the acid hydrolysis (**1**) and (**2**) along with glucose and arabinose but these molar ratios were different from each other. Fraction A_1. colourless needles or leaflets, mp 274–275° from H_2O (a few drops of $CHCl_3$ and MeOH). Fraction A_2: colourless needles, mp 258–260° from aq. MeOH.

Periodate oxidation of bacoside fraction A . Fraction A_1 (300 mg) was oxidized with periodate twice as in the case of crude bacoside A (see above) and the reaction mixture was chromatographed on Si gel to obtain jujubogenin (**4**), colourless needles, mp 248–252° from MeOH (yield: 30.5 mg), and pseudojujubogenin (**6**), colourless needles, mp 273–276° from MeOH (yield. 11.2 mg).

Periodate oxidation of bacoside fraction A_2. Fraction A_2 (300 mg) was oxidized with periodate and hydrolysed with 5% KOH (see above). The final reaction mixture was chromatographed on Si gel to give two substances as the main sapogenins. One of them was identified as (**4**) (yield. 14.5 mg), and the other (**6**), (yield: 22.5 mg).

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