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A NEW SAPOGENIN IN THE SAPONINS OF *ZIZYPHUS JUJUBA, HOVENIA DULCIS* AND *BACOPA MONNIERA**

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Key Word Index—*Zizyphus jujuba*, *Hovenia dulcis*, Rhamnaceae, *Bacopa monniera*, Scrophulariaceae, jujuboside A, jujuboside B, hovenoside G, bacoside A, ebelin lactone

Abstract—Acid hydrolysis of the saponin of the seeds of *Zizyphus jujuba* afforded ebelin lactone, which yielded the sapogenin, jujubogenin, on Smith-de Mayo degradation. The mechanism of conversion of jujubogenin into ebelin lactone has been elucidated. Hovenoside G of *Hovenia dulcis* and bacoside A of *Bacopa monniera* which produce ebelin lactone on acid hydrolysis have also been found to yield jujubogenin on Smith-de Mayo degradation.

"SANZAOREN" (Sansonin in Japanese), the seeds of *Zizyphus jujuba* Mill (Rhamnaceae)[1a] used in Chinese Medicine as a drug for insomnia and sometimes for sleepiness caused by physical emaciation,[1b] contain saponins, jujuboside A and B, from which we[2] recently obtained by the acid hydrolysis ebelin lactone (**1**)[3] as the sapogenin and glucose, rhamnose, arabinose and xylose as the sugars. However, since the saponins showed no evidence of possessing a lactone and a conjugated double bond by the IR and UV spectra, respectively, ebelin lactone must be an artifact produced by the action of acid. Jujuboside A is partially hydrolyzed by the snail enzyme[4] to give jujuboside B.

On Smith-de Mayo degradation[5] which was performed twice, jujuboside B afforded a sapogenin, $C_{30}H_{48}O_4$, m.p. 250–252°, named jujubogenin (**2**) as the main product.

On treatment of the saponin with periodate followed by reduction with $NaBH_4$, a prosapogenin which possesses arabinose as the only sugar was obtained. By the action of sulphuric acid, jujubogenin (**2**) was converted almost quantitatively into ebelin lactone (**1**). The IR and UV spectra of jujubogenin indicate the absence of carbonyl and conjugated system in the molecule, as in the case of the parent saponins. From the above results and the NMR spectral data of jujubogenin compared with those of ebelin lactone, it is sug-

* Part XXXVIII in the series 'Chemical Studies on the Oriental Plant Drugs'. For part XXXVII see KANEDA, M., SAITOH, T., IITAKA, Y. and SHIBATA, S. (1973) *Chem. Pharm. Bull.* (Tokyo) **21**, 1338.

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[1] (a) cf. SATO, J. *On the Chinese Medical Plants* p. 313 (Japan Society for the Promotion of Science, March 1959), OHWI, J. *Flora of Japan* (Revised edn.) p. 876 (Shibundo, Tokyo, 1965), *Icomoglaphia Cormophy-orum-Sinicorum* Tom. II p. 753 (Sci. Publ. Peking 1972).

[1] (b) ZONG-YAO-ZHI, vol. II, p. 448, Drug Research Laboratory, Medical Research Institute, Peking 1961).

[2] SHIBATA, S., NAGAI, Y., TANAKA, O. and DOI, O. (1970) *Phytochemistry* **9**, 677.

[3] EADE, R. A., ROSSLER, L. P., SIMES, H. V. and SIMES, J. J. H. (1965) *Aust. J. Chem.* **18**, 1451.

[4] Suc D'helix pomatia (Industrie Biologique Francaise).

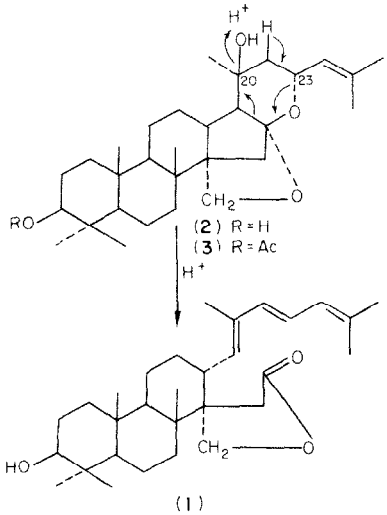
[5] DUGAN, J. J. and DE MAYO, P. (1965) *Can. J. Chem.* **43**, 2033.

gested that jujubogenin must be a dammarane-type triterpene. On acetylation, jujubogenin afforded a monoacetate (**3**), m.p. 248–250°, in which one tertiary hydroxyl (IR 3460 cm^{-1}) remained in free.

The NMR signal of a proton attached to the carbon bearing a hydroxyl which appears at δ 3.16 (*q*) in jujubogenin (**2**) is shifted to δ 4.40 (*q*, *J* 9, 6 Hz) in its monoacetate without any significant change in other NMR signals. Therefore, the secondary hydroxyl of jujubogenin must be located at $C_{(3)}-\beta$ as in ebelin lactone and the tertiary hydroxyl at $C_{(20)}$. Thus two of four oxygen functions of **2** have been assigned to 2 hydroxyls, and the remaining 2 oxygens form ethers or a ketal. The signal at δ 3.99 (*br* s) is assigned to a methylene attached to a primary oxygen function, while δ 4.60 (*s*, *J* 9, 9, 4.5 Hz) to a proton attached to a carbon bearing a secondary oxygen function.

The signal at δ 5.20 (*br d*, *J* 9 Hz) is assigned to proton at $C_{(24)}$ which is converted into a broad singlet when the proton at δ 4.60 is irradiated. This has been rationalized in assigning the proton of δ 4.60 to a position next to a double bond and a methylene.

From the structure of ebelin lactone and the above NMR data, structure (**2**) must represent jujubogenin, and its conversion into ebelin lactone (**1**) would be as follows.



(1)

By the X-ray crystallography of mono-*p*-bromobenzoate of **2**, the absolute configurations at $C_{(20)}$ and $C_{(23)}$ of **2** have been established to be S and R, respectively.* Jujubogenin is a genuine sapogenin, though this has not conclusively been established.

Meanwhile, the root bark of *Hovenia dulcis* (Rhamnaceae) contains, in addition to basic substances,[6] saponins which must closely be related to jujubosides, since these saponins also afforded ebelin lactone on acid hydrolysis. The saponins hovenosides C, D, G, G′ and H were isolated by a droplet counter current chromatography (DCCC)[7] from the butanol-soluble fraction of the non-alkaloidal methanolic extracts.

On treatment with sulphuric acid, hovenoside G, the main saponin of *Hovenia dulcis*, afforded ebelin lactone (**1**) as the major sapogenin, and glucose, arabinose and xylose. The

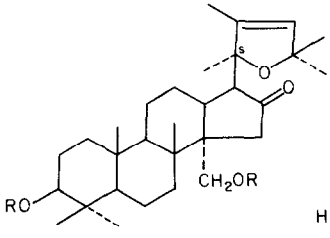
* These details will be reported elsewhere.

[6] TAKAI, M., OGIHARA, Y. and SHIBATA, S. (1973) *Phytochemistry*. In press.

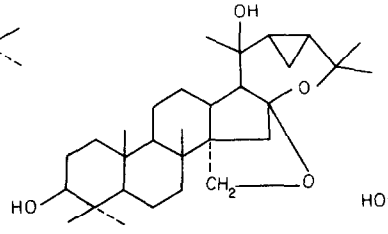
[7] TANIMURA, T., PISANO, J. J., LIU, Y. and BOWMAN, R. L. (1970) *Science* **169**, 54.

saponin shows no carbonyl and conjugated double bond absorptions in the IR and UV spectra, respectively. Hovenoside G was treated twice with periodate and then with alkali to afford a crystalline sapogenin, m.p. 250–252°, which was identified as jujubogenin by mixed m.p. and comparison of spectral data.

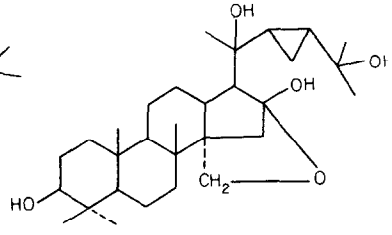
Furthermore, Rastogi *et al.*[8] reported the occurrence of saponins, bacosides A and B in an Indian Medicinal plant, *Bacopa monniera* (Scrophulariaceae). Bacoside A yielded bacogenin A_1 (**4**) and ebelin lactone (**1**),[9] besides glucose and arabinose,[10] on acid hydrolysis. The structure of bacogenin A_1 (**4**) was established by the X-ray crystallography of its dibromoacetate (**5**).[11] Rastogi *et al.* recently proposed two alternative structures, (**6**) and (**7**),[12] for the genuine sapogenin of bacoside A without experimental evidence. On treatment of bacoside A with periodate and alkali as described above, jujubogenin was isolated from the reaction mixture along with another sapogenin whose NMR spectrum is similar to that of jujubogenin and the structure is now being investigated



(**4**) R = H
(**5**) R = $COCH_2Br$



(**6**)



(**7**)

EXPERIMENTAL

The isolation of jujubosides A and B The defatted seeds of *Zizyphus jujuba* Mill were extracted with MeOH The methanolic extracts were dissolved in H_2O and extracted with Et_2O to remove non-glycosidic substances The aq layer was extracted with BuOH and washed with 1% aq KOH From the butanol layer crude saponin was obtained (yield about 0 1%) The saponin fraction was chromatographed on silica gel to obtain jujubosides A and B The former was eluted from the chromatographic column with CH_2Cl_2–MeOH (17 3), and the latter with CH_2Cl_2–MeOH (4 1) Jujubosides A and B were also isolated with DCCC such as described below

Jujuboside A Colourless powder, ν_{max}^{KBr} 3400 (OH), 1100 ~ 1000 cm^{-1} λ_{max}^{EtOH} no absorption above 200 nm δ In d_5-pyridine 0 72 (3H, s, -C<u>H</u>$_3$), 1 13 (9H, s, -C<u>H</u>$_3$ × 3), 1 41 (3H, s, >C(-OH)-C<u>H</u>$_3$), 1 72 (6H *br* s, =C(-C<u>H</u>$_3$)$_2$), 2 50 (1H, *d*, *J* 8), 2 86 (1H, br), 3 16 (1H, br), 4 ~ 5 4 (unassigned), 5 57 (1H, *br d*, *J* 9), 5 97 (1H, *br s*) (Yield 38% of the crude saponin)

Jujuboside B Colourless crystals, ν_{max}^{KBr} 3400 (OH), 1100 ~ 1000 cm^{-1} λ_{max}^{EtOH} no absorption above 200 nm δ In d_5-pyridine 0 73, 1 06, 1 12, 1 15 (3H each, *s*-C<u>H</u>$_3$ × 4), 1 40 (6H, *s*, >C(-O-C<u>H</u>$_3$ × 2), 1 67, 1 72 (3H each, *br* s, >C=C<u>H</u>$_3$), 2 41 (1H, *d*, *J* 8), 2 78 (1H, br), 3 14 (1H, *q*-like, >C-OH-<u>H</u>), 3 5 ~ 5 4 (unassigned), 5 47 (1H, *d*, *J* 8), 5 85 (1H, *br s*) (Yield 25 5% of the crude saponin) On the acid hydrolysis, jujuboside B yielded ebelin lactone (**1**) along with glucose, rhamnose, arabinose and xylose in equivalent molar ratios

Periodate oxidation of jujuboside B Jujuboside B (500 mg) was oxidized with $NaIO_4$ (1 g in 100 ml of 50% aq EtOH) at room temp for 24–48 hr The reaction mixture was refluxed with 5% KOH for 3 hr and then extracted with BuOH(× 2) The final products were chromatographed on silica gel to separate the main sapogenin by elution with benzene–acetone (10 1) The sapogenin, jujubogenin (**2**), was obtained as colourless needles on recrystallization from MeOH (yield 17 mg)

Jujubogenin (**2**) Colourless needles, m p 250 ~ 252° from MeOH ν_{max}^{KBr} 3500, 3300 (OH), 1286, 1007 cm^{-1} λ_{max}^{EtOH} end absorption only $[\alpha]_D^{25}$ – 36° (EtOH, *c* 0 069) δ In $CDCl_3$ 0 77, 0 83, 0 95, 1 10 (3H each, s, tert -C<u>H</u>$_3$ × 4), 1 17 (3H, s, > C-OH-C<u>H</u>$_3$), 1 65, 1 69 (3H each, *br* s, =C<u>H</u>$_3$), 1 96 (1H, *d*, *J* 9Hz), 2 32 (1H, *q*-like), 3 16 (1H, *q*, *J* 10 and 6Hz, -C-OH-<u>H</u>), 3 99 (2<u>H</u>, *br* s, -O-C<u>H</u>$_2$-C=), 4 63 (1H, s, *J* 8, 8 and 4 Hz,

[8] CHATTERJI N, RASTOGI, R P and DHAR, M L (1963) *Indian J Chem* **1**, 212
[9] KURSHRESHTHA, D K and RASTOGI, R P (1973) *Phytochemistry* **12**, 887
[10] CHATTERJI, N, RASTOGI, R P and DHAR, M L (1965) *Indian J Chem* **3**, 24
[11] KAWAI, K, IITAKA, Y, SHIBATA, S, KURSHRISHTHA, D K and RASTOGI, R P (1973) *Acta Cryst* In press
[12] KULSHRESHTHA, D K and RASTOGI, R P (1973) *Phytochemistry* **12**, 2074

-CH_2-C(-O-H)-C(-H)-), 5.20 (1H, br. d, J 8 Hz, >C=C(-H)-C(-H)<), δ in d_5-pyridine: 0.81, 0.98, 1.11, 1.17, 1.33 (3H each, s), 1.68 (6H, br. s), 2.39 (1H, d, J 8 Hz), 2.76 (1H, q-like), 3.36 (1H, t, J 8 Hz), 4.15 (2H, a pair of doublets, J 15 Hz), 5.01 (1H, br.), 5.46 (1H, br. d, J 7 Hz), 5.74 (1H, br. s, -OH). (Found: C, 76.05; H, 10.40. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24.) **2** Afforded ebelin lactone (**1**) almost quantitatively by the hydrolysis with H_2SO_4.

Jujubogenin monoacetate (**3**). Jujubogenin (**2**) (21 mg) was acetylated with Ac_2O and pyridine giving jujubogenin monoacetate (**3**) (9 mg) as colourless needles, m.p. 248 ~ 250° from MeOH. ν_{max}^{KBr} 3400 (OH), 1733, 1243 (OAc), 1027, 1003, 982. ε_{max}^{EtOH}: no absorption above 200 nm. $[\alpha]_D^{25}$ −25 (EtOH, c 0.052). δ in $CDCl_3$: 0.85 (9H, s, tert.-CH_3 × 3), 1.11 (3H, s, tert.-CH_3), 1.18 (3H, s, >C(-OH)-CH_3), 1.66, 1.70 (3H each, br. s, C(-CH_3)=), 1.96 (1H, d, J 9 Hz), 2.03 (3H, s, -OCOCH_3), 2.38 (1H, br.), 3.99 (2H, br. s, >C(-CH_2-O-)), 4.40 (1H, q, J 9 and 7 Hz, -C(-OAc)-H), 4.60 (1H, s, J 9, 9 and 4.5 Hz, -CH_2-O-C(-H)-C(-H)=), 5.19 (1H, br. d, J 9 Hz). (Found: C, 74.51; H, 9.90. Calcd. for $C_{32}H_{50}O_4$: C, 74.67; H, 9.79.)

Isolation of hovenosides. The dried root bark of *Hovenia dulcis* Thunb. (800 g) was defatted with C_6H_6 and extracted with MeOH. The methanolic extracts were dissolved in water and extracted with Et_2O. The aqueous layer was basified with NH_4OH and extracted with $CHCl_3$ to remove the alkaloidal substances. KOH was added to the aq. layer which was extracted with BuOH. The butanolic extracts (29 g), the so-called "saponin fraction" was subjected to a droplet countercurrent chromatography (DCCC) using $CHCl_3$-MeOH-H_2O (5:6:4) to separate it into several saponins, *i.e.* hovenosides C, D, G, G', and H which were named in accordance with increasing Rf in $CHCl_3$-MeOH-H_2O (13:7:2, lower layer). The main saponin, hovenoside G (yield: 25% of "saponin fraction") afforded on hydrolysis with H_2SO_4 ebelin lactone (**1**) and glucose, arabinose and xylose in the ratio 1:1:2.

Hovenoside G. Colourless crystals, m.p. 230 ~ 234° (decomp.) from aq. MeOH. ν_{max}^{KBr} 3400, 1100 ~ 1000 cm^{-1}. ε_{max}^{EtOH}: no absorption above 200 nm. σ in CD_3OD: 0.85 (6H, s, -CMe × 2), 1.03 (3H, s, -CMe), 1.12 (6H, s, -CMe × 2), 1.67, 1.70 (3H each, br. s, =C(-CH_3)), 2.05 (1H, d, J 10 Hz), 2.44 (1H, m), 2.9 ~ 5.0 (unassigned protons), 4.30 (1H, d, J 7 Hz), 5.13 (1H, d, J 7).

Periodate oxidation of hovenoside G. Hovenoside G (520 mg) was oxidized with periodate twice as in the case of jujuboside B (see above) and the reaction mixture was chromatographed on silica gel to obtain the main sapogenin (28 mg) as colourless needles, m.p. 250 ~ 252° from MeOH, which was proved to be identical with jujubogenin (**2**) by IR, NMR, MS, TLC and m.m.p. comparison. Furthermore, the acetate of this sapogenin was proved to be identical with jujubogenin acetate (**3**) (IR, NMR, MS and TLC).

Periodate oxidation of bacoside-A. Bacoside-A (630 mg) was oxidized with periodate and hydrolysed with 5% KOH (see above). The reaction mixture was chromatographed on silica gel to give two substances as the main sapogenins. One of them, colourless needles, m.p. 248 ~ 252°, from MeOH (yield: 9 mg) was identified as jujubogenin (**2**) by the comparison of the spectral data of it and its acetate with those of jujubogenin acetate (**3**). The other sapogenin, colourless needles, m.p. 251 ~ 256° from MeOH (yield: 7 mg), gave a very similar pattern in its NMR spectrum as **2**, but it is obviously different.

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