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A NOVEL PREGNANE DERIVATIVE FROM *SARCOSTEMMA BREVISTIGMA*

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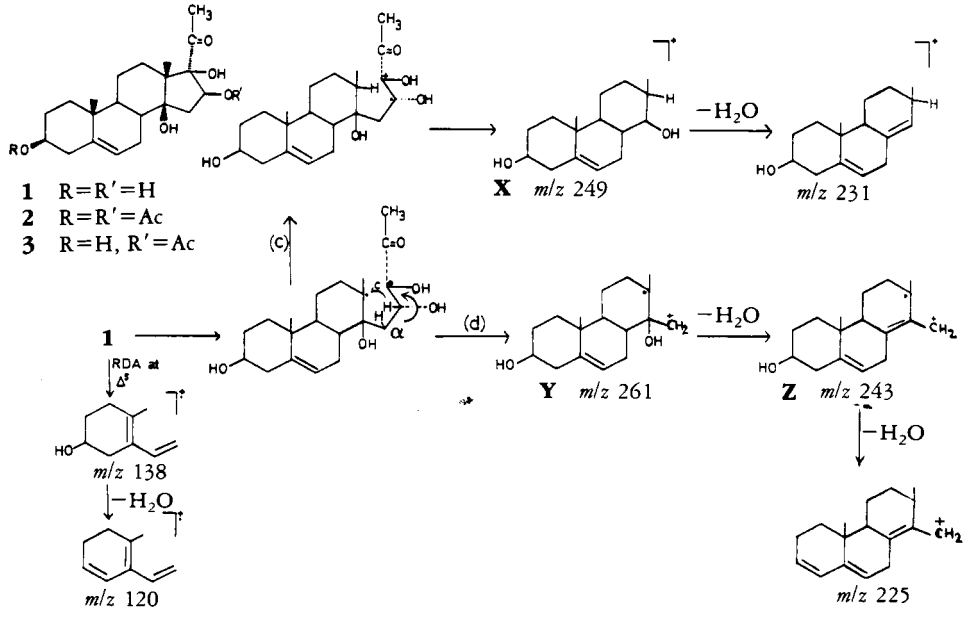
ABSTRACT.—A new pregnane derivative designated as bregenin [**1**] has been isolated from the dried twigs of *Sarcostemma brevistigma*. Chemical and spectroscopic evidence is consistent with the structure 3β,14β,16α,17β tetrahydroxypregn-5-en-20-one for bregenin.

In an earlier chemical investigation of the aerial part of *Sarcostemma brevistigma* W. and A. (Syn. *S. acidium*, Asclepiadaceae), the presence of the novel sugars brevobiose (1), tigmobiose (2), and sarcobiose (3), the ester glycosides brevinine (4) and brevine (5), and the pregnane derivative sarcogenin (6) were reported. The present paper deals with the isolation and structure elucidation of yet another novel pregnane derivative, bregenin [**1**], as a continuation of the studies on the extract of this plant.

RESULTS AND DISCUSSION

Bregenin [**1**] mp 238-240°, $[\alpha]_D$+60° exhibited its $[M]^+$ at *m/z* 364 which is consistent with a molecular formula $C_{21}H_{32}O_5$. Its ir spectrum provided evidence for hydroxyl groups (3350 cm^{-1}), a trisubstituted double bond (800 cm^{-1} and positive tetranitromethane reaction), and a methyl keto function (1670 cm^{-1} and Me deformation band at 1360 cm^{-1}). The presence of a carbonyl group was also shown by its reduction (7) with $NaBH_4$ and by formation of a 2,4-dinitrophenylhydrazone, and its nature as a methyl ketone was supported by a characteristic color reaction with sodium nitroprusside (8).

The molecular formula of bregenin indicated it to be a highly hydroxylated pregnane genin (C_{21}-steroid). Acetylation of **1** with Ac_2O in pyridine afforded two products, a di-*O*-acetyl bregenin [**2**] mp 242°, $[\alpha]_D$ zero and also a mono-*O*-acetyl bregenin



SCHEME 1

[**3**] mp 96°, $[\alpha]_D$+17°. Formation of a diacetyl derivative from **1** indicated it to contain two acetylable hydroxy functions in the molecule. One of these could be at C-3 as evidenced by the mass spectral fragments at *m/z* 138 and 120 characteristic of Δ^5-3-ol arrangement (9), whereas the position of the other acetylable hydroxyl remains to be fixed. As **2** still contained a free hydroxyl group (ir spectrum), an assignment of all the hydroxyl functions in **1** could be arrived at by accounting for the five oxygen atoms in the bregenin molecule. The presence of one oxygen as a keto group and two as acetylatable hydroxyl groups led us to postulate two tertiary hydroxyl groups (unacetylable) in **1** accounting for the remaining two oxygen atoms in the molecule. They presumably could be present at the C-14 and C-17 positions commonly reported in plant pregnanes. The inability of compound **1** to isomerize with hot methanolic KOH substantiated the presence of a C-17 hydroxyl group in this 20-keto pregnane derivative. Reichstein reported (10) that in pregnane derivatives the hydroxyl group at C-14 is always in the β-configuration, and the C-17 hydroxyl group, if present, is also in the β-configuration, resulting in the α-configuration of the two carbon chain at C-17. He also added that the C-14 hydroxyl group and C-20 keto group in a pregnane derivative are hydrogen bonded (9). The negative Cotton effect of **1** is also in conformity with the α-orientation (11) of the methyl keto chain at C-17. On the basis of the above results and those of commonly reported plant pregnanes, the positions and configurations of three of the hydroxyl groups in **1** were postulated as C-3β, C-14β, and C-17β (^{1}H nmr ϕ ms), and the position and configuration of the fourth acetylable hydroxyl group remained to be fixed. The inertness of **2** to sodium periodate, but a positive reaction of **1** to this reagent suggested that the unassigned acetylable hydroxyl function in **1** is in a vicinal diol arrangement which could be either at C-2 or C-4 or C-16. The hydroxyl group at C-15 is precluded as the C-14, C-15 diol does not react with $NaIO_4$ (12). The mass spectral fragments at *m/z* 138 and 120, characteristic of the Δ^5-3-ol system, precluded the presence of a C-2 or C-4 hydroxyl group. These data led to the conclusion that the fourth hydroxyl group is present at the C-16 position, which was also supported by the ms fragments. The *trans* configuration of the C-16 and C-17 hydroxyl groups was derived from the inability of **1** to form an isopropylidene derivative. The β-configuration of the C-17 hydroxyl group already postulated above, thus, led to the conclusion that the C-16 hydroxyl group is in the α configuration. This conclusion is in agreement with the nuclear magnetic double resonance experiments (nmdr).

A detailed analysis of the ms of the pregnane derivative **1** was very helpful in ascertaining the nature, number, and positions of its various groups. The molecular ion peak at *m/z* 364 showed fragmentation in different sequences giving ion peaks at *m/z* 346 (M-H_2O), 328 (M-2H_2O), 313 (328-CH_3), 310 (M-3H_2O), 295 (313-H_2O), 267 (310-MeCO), 249 (267-H_2O) and also peaks at *m/z* 285 (328-MeCO), 267 (285-H_2O) confirming the presence of four hydroxyl groups and a methyl keto chain in **1**.

The diagnostic fragments **X**, **Y**, and **Z** giving peaks at *m/z* 249, 261, and 243 and their fragments showing further losses of H_2O molecules giving peaks at *m/z* 231 (249-H_2O) and 225 (243-H_2O) provide strong support for the vicinal diol arrangement in **1** at C-16 and C-17 (Scheme 1).

The 400 MHz ^{1}H-nmr spectrum of **1** and its acetates not only confirmed that it is a Δ^5-pregn-20-one but also confirmed the position of its hydroxyl groups. The spectrum of **1** contained signals for two angular methyl groups, a keto methyl group, and an olefinic proton in addition to a one-proton multiplet centered at δ 3.57 and a one proton double doublet (J= 10 and 4.5 Hz) at δ 3.73 attributable to the methine protons at C-3 and C-16, respectively. The large coupling constant (J= 10 Hz) was typical of an axial methine proton at C-16 confirming the α-configuration of the C-16 hydroxyl group. This assignment is also confirmed by nmdr experiments. Irradiation of the multiplet at

δ 1.90 corresponding to the C-15 methylene croup caused a collapse of the multiplicity of the C-16 methine proton multiplet to a singlet at δ 3.73. Conversely, irradiation of the C-16 carbinol methine proton double doublet at δ 3.73 resulted in the collapse of the methylene group multiplet into a broad singlet at δ 1.90.

The 90 MHz ^{1}H-nmr spectrum of diacetate **2** is also in full support to the proposed structure. As expected the spectrum consists of five three-proton singlets, two for the tertiary angular methyl groups of the steroid moiety, two for acetoxy groups at the C-3 and C-16 positions, and one for the C-17 methyl keto side chain. The spectrum also contained a double doublet (J= 10 and 4.5 Hz) at δ 4.53 and a multiplet at δ 4.56 attributable to the secondary carbinol methine protons at C-16 and C-3, respectively. The ^{1}H-nmr spectrum of **2** also contained a two-proton multiplet at δ 2.05. Irradiation of this multiplet collapsed the C-16 methine proton at δ 4.53, while irradiation of the signal at δ 4.53 led to the collapse of the methylene group multiplet into a broad singlet at δ 2.05.

The mass spectrum of **2** is in full support of the derived structure. The highest mass ion peak at *m/z* 388 (M-CH_3COOH), undergoes further fragmentation giving ion peaks at *m/z* 370 (388-H_2O), 352 (388-2H_2O), 328 (388-CH_3COOH), 313 (328-CH_3), 310 (328-H_2O), 295 (313-H_2O), 285 (328-$COMe_3$), 267 (285-H_2O), and 249 (267-H_2O) again confirming the presence of two hydroxyl groups and two acetyl groups in **2** besides the retro-Diels Alder fragments at *m/z* 120 and 105.

During the acetylation, the partially hydrolyzed product, mono-*O*-acetyl bregenin [**3**] mp 96°, [α]D+17° was also obtained. A closer analysis of the 90 MHz ^{1}H-nmr spectrum of the mono-*O*-acetyl bregenin [**3**] indicated it to be 16-*O*-acetyl bregenin as its spectrum consists of four three proton singlets: two for the tertiary angular methyl group, one for the acetoxy group presumably at C-16 and one for the C-17 methyl keto chain. The assignment of the acetoxy group to the C-16 position is confirmed by irradiation of the methine proton multiplet centered at δ 4.55, which caused a collapse of the methylene multiplet into a broad singlet at δ 2.00.

The mass spectrum of **3** exhibited its highest mass ion peak at *m/z* 352 (M-3H_2O). Other fragment ion peaks appearing in lower mass region were due to the loss of a molecule of HOAc, three molecules of H_2O, and the keto methyl side chain, giving fragment ion peaks at *m/z* 352 (M-3H_2O), 346 (M-CH_3COOH), 328 (346-H_2O), 313 (328-CH_3(, 310 (328-H_2O), 295 (313-H_2O), 292 (346-3H_2O), 285 (328-COMe), 277 (313-2H_2O), 292 (346-3H_2O), 285 (328-COMe), 277 (313-2H_2O), 267 (285-H_2O), and 249 (292-COMe) besides the retro-Diels-Alder fragment ion peaks at *m/z* 120 and 105.

On the basis of the above chemical and spectroscopic evidence, the structure of bregenin [**1**] was found to be 3β,14β,16α,17β-tetrahydroxypregn-5-en-20-one.

EXPERIMENTAL

The general procedures were the same as those reported recently (6). The plant *S. brevistigma* was collected in Rajasthan and identified by Dr. S.L. Kapoor, National Botanical Research Institute, Lucknow; a voucher specimen, Herbarium No. 16267, is preserved at the institute.

EXTRACTION AND ISOLATION.—The extraction of twigs of *S. brevistigma* was described earlier (13) by the method used for pregnane glycosides (14), and a genin mixture was obtained by mild acid hydrolysis of combined Et_2O and $CHCl_3$ extracts. Repeated column chromatography of this mixture (7.35 g) over Si gel using $CHCl_3$/MeOH as eluent afforded bregenin [**1**] (52 mg).

BREGENIN [**1**].—Mp 238-240°, $[\alpha]^{25}D$+60° (MeOH, *c* 0.11); (Found: C, 69.10; H, 8.20, $C_{21}H_{32}O_5$ required c, 69.23; H, 8.79%). It gave positive color tests with 2,4-dinitrophenyl hydrazine, tetranitromethane, and sodium nitroprusside. Ir spectrum ν max (KBr) cm^{-1} 3350, 2930, 1700, 1670, 1445, 1380, 1360, 1282, 1205, 1038, 950, 895, 865, 800; $[\alpha]_{280}$-33421 86; ^{1}H nmr ($CDCl_3$) 5.36 (1H, m, H-6), 3.73 (1H, dd, J= 10 and 5 Hz, H-16), 3.57 (1H, m, H-3), 2.26 (3H, s, Ac), 1.90 (2H,

m, H-15), 1.27 (3H, s, 18-Me) and 1.18 (3H, s, 19-Me); ms *m/z* (rel. int.) $[M]^+$ 364 (0.2), 346 (90), 331 (42), 328 (75), 313 (85), 310 (15), 295 (45), 285 (32), 276 (28), 267 (28), 261 (12), 253 (15), 249 (20), 243 (18), 233 (30), 231 (25), 225 (20), 215 (20), 208 (35), 180 (42), 147 (45), 138 (15), 120 (50), 81 (25), 67 (12), 55 (28), 43 (100).

$NaIO_4$ OXIDATION OF COMPOUND **1**.—Compound **1** (4 mg) in MeOH (0.4 ml) was oxidized with $NaIO_4$ (10 mg) and kept at room temperature for 4 h. After the usual work-up, it yielded one spot of lower Rf value than compound **1**.

$NaBH_4$ REDUCTION OF COMPOUND **1**.—Substance **1** (4 mg) in MeOH (0.4 ml) was reduced with $NaBH_4$ (4 mg) and kept at room temperature for 2 h. After the usual work-up, it gave two spots (85:15) of close mobility at a lower Rf value than compound **1**.

ACETYLATION OF COMPOUND **1**.—Compound **1** (20 mg) on acetylation with pyridine (1.1 ml) and Ac_2O (0.4 ml) at 100° for 2 h afforded two products (tlc) **2** and **3** of higher mobility than **1**. These products were separated on a Si gel column. Compound **2**, mp 242-246°, $[\alpha]^{25}D$ zero; ^1H nmr ($CDCl_3$) 5.32 (1H, m, H-6), 4.56 (1H, m, H-3), 4.53 (1H, dd, $J=10$ and 4.5 Hz, H-16), 2.05 (2H, m, H-15), 2.15 (3H, s, Ac), 1.99 (3H, s, OAc), 1.90 (3H, s, OAc), 1.22 (3H, s, 18-CH_3), and 1.10 (3H, s, 19-CH_3); ms *m/z* (rel. int.) $[M]^+$ (not observed), 388 (50), 370 (10), 352 (22), 328 (45), 313 (25), 310 (20), 295 (22), 285 (0.5), 277 (12), 267 (0.8), 249 (20), 120 (95), 105 (22), 43 (100). Compound **3**, mp 96-98°, $[\alpha]^{25}D+17°$, (MeOH, *c* 0.13); ^1H nmr ($CDCl_3$) 5.35 (1H, m, H-6), 4.55 (1H, m, H-16), 3.65 (1H, m, H-3), 2.00 (2H, m, H-15), 2.21 (3H, s, Ac), 2.06 (3H, s, OAc), 1.24 (3H, s, 18-CH_3), and 1.15 (3H, s, 19-CH_3); ms *m/z* (rel. int.) $[M]^+$ (not observed), 352 (18), 346 (12), 328 (100), 313 (55), 310 (60), 295 (25), 292 (82), 285 (10), 277 (45), 267 (20), 249 (62), 209 (25), 162 (52), 120 (60), 105 (12).

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