(450 mg) sterol fractions. The following constituents were tentatively identified by GLC and GC–MS as described above: cycloartanol (approximately 11%), cycloartenol (75%) and 24-methylenecycloartanol (4%) in the 4,4-dimethylsterol fraction; 31-norlanostenol and 4α-methylzymostenol (unresolved, 25%), lophenol (43%), 31-norcycloartenol (12%), and gramisterol and cycloeucalenol (unresolved, 3%) in the 4-monomethylsterol fraction; and cholesterol (14%), cholest-7-enol (1%), campesterol (8%), stigmasterol (trace), sitosterol (68%), and 28-isofucosterol (9%) in the 4-desmethylsterol fraction.

Allium fistulosum var. *caespitosum* Makino (shallot) was reported to contain cholesterol, campesterol, stigmasterol, and sitosterol in the plant separated from the root [12].

Preparative Si gel TLC as described above of the unsaponifiable material (2.44 g) of the lipid (13 g), extracted from the milled and dried plant (1250 g), separated from the root, by CH_2Cl_2, gave 4,4-dimethyl- (240 mg), 4-monomethyl- (290 mg) and 4-desmethyl- (370 mg) sterol fractions. Tentative identification of the sterols in each fraction was made by GLC and GC–MS: cycloartanol (approximately 33%), cycloartenol (39%) and 24-methylenecycloartanol (24%) in the 4,4-dimethylsterol fraction; 31-norlanostenol and 4α-methylzymostenol (unresolved, 24%), lophenol (30%), 31-norcycloartenol (15%), gramisterol and cycloeucalenol (unresolved, 4%), and citrostadienol (trace) in the 4-monomethylsterol fraction; and cholesterol (32%), cholest-7-enol (2%), campesterol (4%), stigmasterol (1%), sitosterol (48%), and 28-isofucosterol (13%) in the 4-desmethylsterol fraction.

A marked similarity in the composition of the sterol mixture is observed among these three Liliaceae plants. The C-24 unalkylated sterols, especially those with a saturated side chain, were abundant in the 4,4-dimethyl- and 4-monomethyl-sterol fractions, and a remarkable amount of cholesterol was found in the 4-desmethylsterol fraction. This composition pattern of sterols is markedly different from those observed for the seed sterols of a number of other higher plants previously studied in this laboratory [1, 5, 13, 14]: the 4-monomethylsterol fraction consists almost exclusively of C-24 alkylated sterols and the 4-desmethylsterol fraction contains at most a very small amount of cholesterol. One exception is red pepper, *Capsicum annuum* (Solanaceae), [5], which shows a sterol composition similar to that of the three Liliaceae plants. It is now well known that liliaceous and solanaceous plants contain a notable amount of C_{27} steroidal sapogenins and alkaloids and that cholesterol is the main biosynthetic precursor of these sapogenins and alkaloids [15–17]. These facts being taken into account, it seems to be of special interest that cholesterol occurs in an unusually large proportion in the 4-desmethylsterol fractions of the three Liliaceae plants and a Solanaceae plant, *C. annuum* [5].

A considerable amount of cholest-7-enol as well as cholesterol was found in the 4-desmethylsterol fraction of *C. indivisa* seeds. This appears to be the first record of such a high content of this sterol in higher plants.

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BACOGENIN-A_3: A NEW SAPOGENIN FROM *BACOPA MONNIERA*

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Key Word Index—*Bacopa monniera*; Scrophulanaceae; bacogenin A_3; triterpenoids.

Abstract—The structure of bacogenin A_3, one of the sapogenins of the bacosides has been established by various chemical and spectral methods.

INTRODUCTION

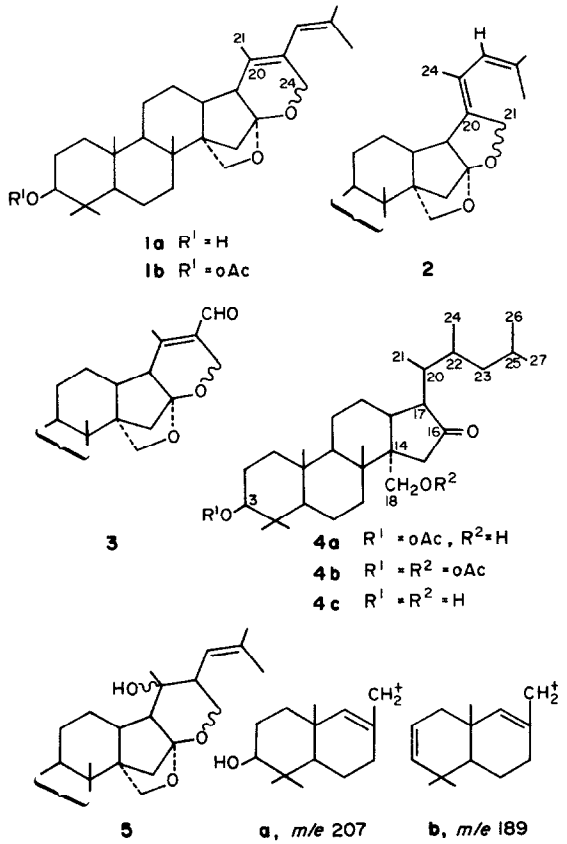
The acid hydrolysis of the crystalline saponins of *Bacopa monniera* gave rise to a mixture of four bacogenins [1]. Bacogenin A_4 was identified as ebelin lactone [2]

whereas bacogenins A_1 and A_2 were characterized as new epimeric genins [3–6]. The present communication deals with the structure elucidation of bacogenin A_3.

RESULTS AND DISCUSSION

Bacogenin A_3, $C_{30}H_{46}O_3$ (M^+ 454), IR absorption at 3344 cm^{-1} (OH), exhibited NMR signals for four tertiary C—Me (0.78, 0.84, 0.98, 1.03 ppm), three vinylic C—Me (6H *d*, *J* 1.5 Hz, 1.53 ppm and 3H *d*, *J* 1.5 Hz, 1.76 ppm), two $—CH_2—O—$ (broad *s*, 4.1 ppm), one —CHO— (*m*, 3.21 ppm) and a vinylic H (*m*, 5.43 ppm). Its UV spectrum indicated the presence of a diene chromophore (230 nm, ε 9630).

Bacogenin A_3 furnished a monoacetate (**1b**), $C_{32}H_{48}O_4$ which showed IR absorption at 1730, 1260 cm^{-1} (—CCO Me). The absence of OH group in the IR spectrum and the presence of a singlet due to an acetoxymethyl at 2.05 ppm with a concurrent shift of the carbinolic proton signal to 4.48 ppm (from 3.21 ppm) in the NMR spectrum, indicated the presence of only one secondary OH group in the molecule. The two inert oxygens were, therefore, ethereal in nature and each must be linked to a methylene group which appeared responsible for a 4H broad singlet at 4.1 ppm.



The ozonolysis of **1b** in the presence of pyridine led to a selective cleavage of one of the double bonds to give a trinoraldehyde (**3**), $C_{29}H_{42}O_5$ (M^+ 470), conjugated to with a tetrasubstituted double bond, (1670, 1635 cm^{-1}; 243 nm (Σ 1150). Its NMR spectrum showed a sharp singlet at 9.96 ppm for an aldehydic proton which demonstrated the presence of a —C=C—CH=C $(Me)_2$ group in bacogenin A_3.

Compound **1b**, on catalytic hydrogenation, yielded a hexahydro product (**4a**), $C_{32}H_{54}O_4$ (M^+ 502), which showed OH absorption (3350 cm^{-1}) in the IR but was transparent in the UV. In the NMR spectrum only one of the $—CH_2—O—$ groups, manifested itself by an AB quartet (*J* 12 Hz) at 3.96 ppm, which shifted to 4.43 ppm in its acetylated product (**4b**) with the appearance of an additional acetoxymethyl singlet at 1.96 ppm. The hexahydro product (**4a**), therefore contained a $—CH_2OH$ group on a fully substituted carbon.

The saponification of compound **4b** furnished product **4c**, $C_{30}H_{52}O_3$ (M^+ 460) whose IR spectrum displayed the presence of OH (3344 cm^{-1}) and a cyclopentanone ring (1727 cm^{-1}) in the molecule. The NMR spectrum was devoid of any vinylic H and vinylic Me signals but showed four tertiary C—Me (0.80, 0.90, 1.00, 1.10 ppm), four secondary Me (*d*, *J* 7 Hz, 0.85 ppm), $—CH_2—CO—$ (AB *q*, *J* 16 Hz, 2.18 ppm), —CH—OH (*m*, 3.24 ppm), CH_2OH (AB*q* *J* 12 Hz, 4.0 ppm). The position and splitting pattern of the NMR signals due to four tertiary C—Me, —CHOH and $—CH_2OH$ were very similar to those of bacogenin A_2 (**5**) and it was, therefore, inferred to possess the same skeleton (dammarane type) with identical placement of —CHOH, CH_2OH, C=O groups and the side chain at C-3 (β), C-18 (α), C-16 and C-17 (β) respectively. The AB quartet (*J* 16 Hz) centred at 2.18 ppm, could thus be assigned to the C-15 methylene protons.

Further, the MS of **4c** displayed an M^+ peak at *m/e* 460 besides other prominent peaks at *m/e* 442 ($M-H_2O$), 429 ($M-CH_2OH$), 411 ($M-H_2O—CH_2OH$), 348 (M-side chain + H), 330 ($348-H_2O$), 207 (ion *a*), 189 (ion *b*) and 113 (side chain). The abundant ion *m/e* 348 arising by the loss of the C_8 side chain by McLafferty rearrangement confirmed the relative positions of the side chain and the C=O group. The formation of fragment ions *a* and *b* proved that the primary alcohol group could be accommodated only at C-18 in the dammarane skeleton.

The generation of a carbonyl group, a CH_2OH and a secondary Me in the hexahydro product (**4a**) clearly demonstrated the presence of a cyclic ketal in bacogenin A_3, which opened up on hydrogenation with the concurrent hydrogenolysis of a vinylic $—CH_2—O—$ group. The other three secondary Me groups must have arisen from the three vinylic Me groups in bacogenin A_3. The vinylic $—CH_2O—$ group, moreover must be located on the tetrasubstituted double bond of the diene system since it remained intact after ozonolysis of bacogenin A_3 acetate (**1b**) and appeared as a singlet at 4.15 ppm in the NMR of the aldehyde (**3**). Thus, the hexahydro product (**4a**) carried the side chain, $—\overset{\overset{Me}{|}}{\underset{\underset{Me}{|}}{C}}—CH_2—CH(Me)_2$.

The reconstruction of the diene side chain and a ketal from the hexahydro product (**4c**) would lead to two structures **1a** or **2** for bacogenin A_3. Structure **2** was, however, ruled out on the ground that in none of the bacogenins does C-21 bear an oxygen function. Bacogenin A_3 would, therefore, be represented by structure **1a**.

It may be mentioned that recently hydroxy bacogenin A_3 (**5**) has been isolated from bacoide A by Prof. S. Shibata (personal communication). The formation of **1a** and **5a** from which bacogenins A_1 and A_2 could be

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derived, lends further support to the proposed structure of genuine bacogenin [2].

EXPERIMENTAL

All mp's are uncorrected. NMR spectra were recorded on a 60MH$_3$ machine in $CDCl_3$ unless otherwise stated.

Bacogenin A_3 (**1a**). M.p. 190° ν_{max}^{KBr}: 3344, 2907, 2825, 1630, 1450, 1380, 1276, 1221, 1027, 1000, 940, 905, 835 cm^{-1}, λ_{max}^{EtOH} 230 nm (ε 9630), NMR (ppm): 0.78, 0.84, 0.98, 1.03 (H each, *s*, 4 C—Me), 1.53 (6H, *d*, *J* 1.5 Hz, 2 C=C—Me), 1.76 (3H, *d*, *J* 1.5 Hz, C=C—Me), 3.21 (1H, *m*, —CH—O—), 4.1 (4H, broad *s*, 2 —CH_2—O—), 5.43 (1H, *m*, C=C—H). MS: *m/e* 454 (M$^+$), 439 (M-15), 436 (M-18), 411, 334, 318, 316, 301, 274, 257, 241, 207, 189, 175, 173, 161, 149, 135, 121. (Found: C, 79.13; H, 10.45, $C_{30}H_{46}O_3$ requires C, 79.27: H, 10.20%).

Acetyl bacogenin A_3 (**1b**): A mixture of bacogenin A_3 (250 mg), C_5H_5N (2.5 ml) and Ac_2O (2.5 ml) was allowed to stand overnight. After usual work up, the product was crystallized from MeOH, mp 220°, λ_{max}^{EtOH}: 235 nm NMR (ppm): 0.86 (9H, *s*, 3 C—Me), 1.05 (3H *s*, C—Me), 1.53 (6H, *d*, *J* 1.5 Hz, 2 C=C—Me), 1.78 (3H, *d*, *J* 1.5 Hz, C=C—Me), 2.05 (3H, *s*, OCO Me), 4.1 (4H, broad *s*, 2 —CH_2—O—), 4.48 (1H, *m*, —CHOAc), 5.45 (1H, *m*, —C=C—H). MS: *m/e* (M$^+$ absent), 316, 301, 273, 257, 241, 203, 189, 175, 173, 161, 149, 147, 135, 121.

Ozonolysis of **1b**: A soln of **1b** (100 mg) in CH_2Cl_2 (10 ml) containing C_5H_5N (0.02 ml) was saturated with ozonized oxygen at −80° for 45 min. The solvent was evap. off and the product (**3**) crystallized from MeOH as colourless needles, mp 246°. ν_{max}^{KBr}: 1730, 1670, 1635, 1265, 775 cm^{-1}. λ_{max}^{EtOH}: 243 nm (ε 1150). NMR (ppm): 0.86 (9H, *s*, 3 C—Me), 1.05 (3H, *s*, C—Me), 2.05 (3H, *s*, OCOMe), 2.15 (3H, broad *s*, C=C—Me), 4.15 (2H, *s*, —CH_2O—), 4.5 (2H, AB*q* *J* 14 Hz, —CH_2O— and 1H, *m*, CHOAc), 9.96 (1H, *s*, —CHO), MS: *m/e* 470 (M$^+$), 189, 175, 161, 149, 135, 121, 107, 93, 81, 69.

Hydrogenation of **1b**. Acetylbacogenin A_3 (**1b**), 125 mg, was hydrogenated over Adam's catalyst in $CHCl_3$–EtOAc (1:1, 10 ml) for 4 hr. The product was purified by PLC on Si gel with C_6H_6 – MeOH (96:4) and crystallized from C_6H_6: petrol as colourless needles (**4a**), mp 145° (100 mg), ν_{max}^{KBr}: 3350, 1725, 1250 cm^{-1}. NMR (ppm): 0.85 (9H, *s*, 3 C—Me), 1.08 (3H, *s*, C—Me), 0.85 (12H, *d*, *J* 7Hz, 4 —CHMe), 2.03 (3H, *s*, OCOMe), 2.18 (2H, AB*q*, *J* 16 Hz, —CH_2CO—), 3.96 (2H, AB*q*, *J* 12 Hz, —CH_2OH), 4.48 (1H, *q*, *J* 5 and 9 Hz, —CHOAc), MS. *m/e* 502 (M$^+$), 472, 443, 428, 412, 399, 390 (M-side chain + H), 372, (390–H_2O), 357, 339, 333, 330, 315, 312, 297, 262, 205, 203, 189, 135, 121, 109, 107, 95, 93. The product (**4a**, 50 mg) was acetylated with Ac_2O–C_5H_5N to furnish a diacetate (**4b**, 38 mg) ν_{max}^{KBr}. 1727, 1244 cm^{-1}; NMR (ppm): 1.96, 2.03, (3H each, *s*, 2 OCOMe), 4.43 (2H, AB*q*, *J* 12 Hz, —CH_2OAc), 4.55 (1H, *m*, —CHOAc). The diacetate (**4b**, 60 mg) was saponified with 2M ethanolic KOH at room temp. for 16 hr. The deacetylated product (**4c**, 45 mg) was obtained as a colourless powder. ν_{max}^{KBr}: 3344, 1727 cm^{-1}. NMR 0.80, 0.90, 1.00, 1.10 (3H each, *s*, 4 C—Me). 0.85 (12H, *d*, *J* 7 Hz, 4 CH—Me), 2.18 (2H, AB*q*, *J* 16 Hz, —CH_2CO—), 3.24 (1H, *m*, CHOH), 4.0 (2H, AB*q*, *J* 12 Hz, —CH_2OH). MS: *m/e* 460 (M$^+$), 442 (M-18), 429 (M-CH_2OH), 411, 375, 348 (M-side chain + H), 330, 315, 297, 284, 279, 256, 213, 207, 189, 185, 149, 141, 113 (side chain), 99, 97, 85. (Found: C, 78.32; H, 11.60. $C_{30}H_{52}O_3$ requires C, 78.20; H, 11.37%).

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CAJANONE: AN ANTIFUNGAL ISOFLAVANONE FROM *CAJANUS CAJAN*

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Key Word Index *Cajanus cajan*; Leguminosae; pigeon pea; isoflavanone; antifungal

Plant. Pigeon pea, *Cajanus cajan* (Millsp.). *Source*: International Institute of Tropical Agriculture, Ibadan, Nigeria.

Present work. Cajanone (M$^+$422, $C_{25}H_{26}O_6$) is the major phenolic compound of the roots of pigeon pea, of which it comprises *ca* 0.14% dry weight. Its 60 MHz NMR spectrum shows signals for one dimethylchromene group (6.61δ, *d*, *J* 12 Hz, H_α, 5.49δ, *d*, *J* 12 Hz, H_β, 1.42δ, *s*, 2 Me's), one isopentenyl group (3.27δ, *bd*, —CH_2—, *ca* 5.4δ, *t*, —CH=, 1.73, 1.74δ, *s*, *s*, Me's), a chelated hydroxyl group (12.10δ, *s*) and three aromatic protons (7.10δ, *s*, 6.44δ, *s*, 5.95δ, *s*). These data, together with the UV spectrum, λ_{max}^{EtOH} 225 *sh* (log ε 4.11), 273 (4.30), 293 (4.05), 306 *sh* (3.87), suggest a flavanone or isoflavanone structure; absence from the NMR spectrum of the signal near 2.8δ characteristic of the C-3 proton of flavanones [1] and the presence of a one-proton triplet (*J* 6 Hz) for H-3 at 3.95δ show that cajanone is an isoflavanone. The C-2 protons appear as a complex multiplet at 4.6–4.8δ.

Since the NMR signals for all three aromatic protons are singlets, two of these protons occupy *para* positions in ring B. The MS shows retro-Diels–Alder fragmentation giving rise to *m/e* 218 (8.9%), 219 (100%) and 204 (9.2%). In view of the NMR data, the fragment at *m/e* 204 must incorporate ring B. This fragment loses C_4H_7 to yield *m/e* 149 (metastable ion at *m/e* 108.8) and therefore contains the isopentenyl group. The ring B susbtituents are now seen to be the isopentenyl group and two hydroxyl groups, one of these being adjacent to the isoptentenyl