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THE STRUCTURE OF CALIBAGENIN

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Key Word Index—*Calibanus hookerii*; Liliaceae; sacamecate; steroidal sapogenins; calibagenin.

Abstract—The main sapogenin of *Calibanus hookerii*, calibagenin, $C_{27}H_{46}O_3$, is a new steroidal sapogenin with an open side chain. The structure has been determined by chemical and spectrometric studies as cholest-5-en-3β,16ξ,22ξ-triol. The phytochemical relationships of open side chain sapogenins are discussed.

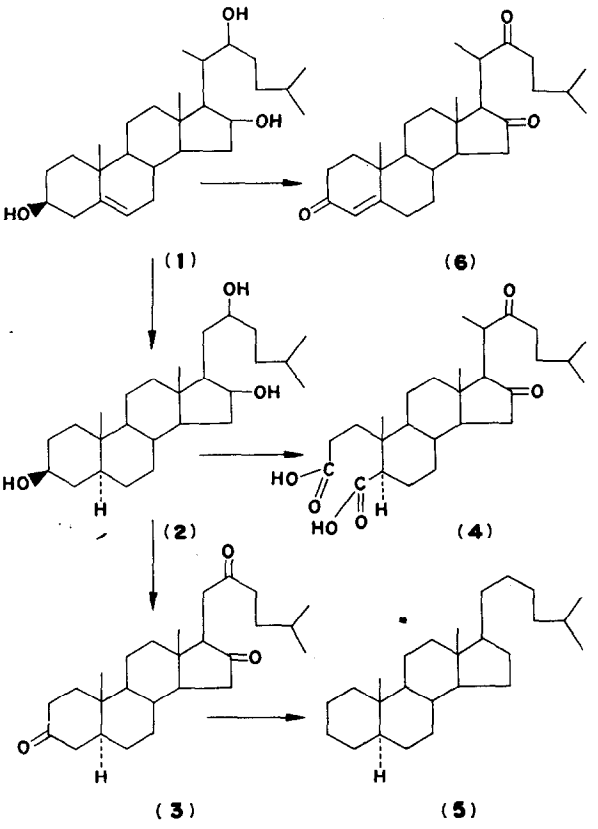
INTRODUCTION

"Sacamecate" is the Mexican name for *Calibanus hookerii*, Liliaceae, a very rare plant found in desert zones of central Mexico. The gigantic rhizomes foam in water due to the saponins they contain and they are used for washing by the peasants in the country. The main saponin, calibanin, contains a steroidal aglycone, calibagenin, which has an open side chain, a double bond and three hydroxyl groups [1]. The present paper reports the elucidation of the structure of calibagenin.

RESULTS AND DISCUSSION

Calibagenin (**1**) was isolated and purified by PLC. The formula was determined by analysis and confirmed by high resolution mass spectrometry (Observed MW: 418.3441; calc MW for $C_{27}H_{46}O_3$: 418·3447). The IR spectrum showed double bond (1625, 840 and 826 cm^{-1}) and OH-group absorptions (3350 cm^{-1}). Calibagenin (**1**) absorbed 1 mol of hydrogen during catalytic hydrogenation. The NMR spectrum of the sapogenin showed only 1 vinylic proton at δ 5·33 ppm which corresponds to a Δ^5 double bond. The NMR signals at 0·907 ppm (C_{18}) and 1·001 ppm (C-19) of calibagenin (**1**) were at 0·855 and 0·91 ppm respectively for dihydrocalibagenin (**2**). Calibagenin gave a tri-*m*-bromobenzoate and all 3 OH groups were secondary since a triketone (**3**) was obtained by oxidation of dihydrocalibagenin (**2**).

The NMR spectrum of calibagenin triacetate showed 3 acetoxy groups as a singlet at 2·055 ppm.



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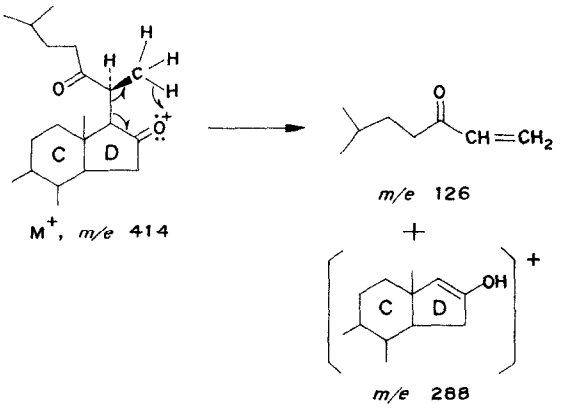
The angular methyl groups appeared at 0·91 ppm (C-18) and 1·03 ppm (C-19) and the calc. values according to Zürcher [2] are 0·909 and 1·08 ppm respectively. Three signals were also observed at 4·58, 4·73 and 4·83 ppm corresponding to the protons on the 3 carbon atoms with the acetate groups. The NMR spectrum of calibagenin (**1**) showed 3 protons resonating at 3·11, 3·56 and 4·36 ppm which were identified as the protons bound to the carbon atoms with secondary alcohol groups (C-22, C-16, C-3).

Oppenauer oxidation of calibagenin (**1**) gave an α,β-unsaturated triketone (**6**). The IR spectrum had absorptions corresponding to a 5-membered ring ketone (1736 cm^{-1}), an acyclic ketone (1717 cm^{-1}) and an α,β-unsaturated cyclic ketone (1670 and 1618 cm^{-1}). The latter group was also revealed by the UV spectrum (λ max 245 nm). Oppenauer oxidation with a longer reflux time gave a polyene hydrocarbon with λ max at 236 nm. The calculated value is 234 nm for a $\Delta^{3,5}$ or $\Delta^{16,20(22)}$ steroid diene and one possible structure for this compound is cholesta-3,5,16,20(22)-tetraene. Controlled Jones oxidation of dihydrocalibagenin (**2**) gave a saturated triketone (**3**) whose IR spectrum showed 2 bands for carbonyl groups at 1713 cm^{-1} (6-membered ring ketone and aliphatic ketone) and 1738 cm^{-1} (5-membered ring ketone). With an excess of Jones reagent dihydrocalibagenin gave a diketodicarboxylic acid (**4**) which was identified by chemical analysis, neutralization equivalent, IR and MS. The IR spectrum showed a broad band from 3525 to 2850 cm^{-1} typical of a carboxylic acid and absorptions at 1700, 1713 cm^{-1} (aliphatic ketone) and 1738 cm^{-1} (5-membered ring ketone). Wolff–Kishner reduction of the triketone (**3**) gave an hydrocarbon (**5**) identified as cholestane by mp and IR.

Calibagenin precipitated with digitonin, showing that one hydroxyl was in the 3β position and periodic acid oxidation was negative showing the absence of a vicinal glycol. The IR of (**3**) and (**4**) showed the presence of a ketone group at C-16 (α-substituted 5-membered ring ketone). This position of the ketone group was confirmed by MS of **3**. The observed fragment at *m/e* 288 was formed from the molecular ion (*m/e* 414) by rearrangement of the C-16 carbonyl group with the transfer of one H from the C-21 methyl group (Scheme 1) [3–5]. A McLafferty rearrangement of the molecular ion



Scheme 1. Mass fragmentation of cholestane-3,16,22-trione side chain.

(*m/e* 414) with the C-22 carbonyl group and transfer of one H from C-25 gave the fragment at *m/e* 358 with the loss of 56 mass units [CH_2=C(Me)$_2$]. The fragment at *m/e* 273 was produced by loss of one methyl group from *m/e* 288. The mass spectrum of **4** showed peaks at *m/e* 99 and 231. The *m/e* 99 fragment came from an α-elimination in the side chain between C-20 and C-22. The peak of *m/e* 231 was formed by elimination of the whole side chain from the *m/e* 358 ion. This fragmentation was confirmed by the metastable ion at 149 (calc. 149·05). The compound **4** also gave a peak at *m/e* 127 which corresponded to the whole side chain.

The mass spectrum of calibagenin showed a base peak at *m/e* 300. The molecular ion lost one molecule of water to give an *m/e* 400 ion. This fragment lost 100 mass units by dehydration at C-16 and fragmentation of the side chain between C-20 and C-22 with the transfer of one H atom from the side chain to the nucleus. The metastable ion was found at *m/e* 225 (calc. 225).

The results presented in this report establish the structure of calibagenin (**1**) as cholest-5-en-3β,16ξ,22ξ-triol. From the biosynthetic viewpoint calibagenin represents an intermediate stage between cholest-5-en-3,22-diol, the sapogenin of *Narthecium ossifragum* [6] (Liliaceae) and kryptogenin (3β,26-dihydroxycholest-5-en-16,22-dione) which has been isolated from *Trillium erectum* (Liliaceae) [7] and from *Dioscorea mexicana* (Dioscoreaceae) [8]. The presence of these open side chain steroidal sapogenins of varying oxidation level in Liliaceae is noteworthy but also striking is

the fact that the more oxygenated open chain sapogenins are present in very big and old rhizomes which take many years to grow, like *C. hookerii* and *D. mexicana*, despite the fact that these plants belong to different families.

EXPERIMENTAL

Calibagenin (**1**) was obtained by the method in [1] and was purified by PLC on Si gel G with $CHCl_3$–MeOH–Me_2CO (9:1:1). It was crystallized from MeOH–H_2O as needles, mp 195–196°, $(\alpha)_D^{20} - 56°$ ($CHCl_3$) (Found: C, 77·28; H, 11·34; O, 11·51. $C_{27}H_{46}O_3$ requires: C, 77·45; H, 11·09; O, 11·46). No. of active hydrogens (Zerewitinof) 0·71% = 2·96 H_2 atoms. IR(KBr): 3350, 2955, 2855, 1625, 1462, 1440, 1375, 1330, 1163, 1070, 1033, 972, 950, 900, 840, 826, 650 cm^{-1}. NMR (60 MHz, $CDCl_3$): δ 0·84 (6H, *d*, J = 4 Hz, C-26 and C-27), 0·907 (3H, *s*, C-18), 1·01 (3H, *s*, C-19), 1·25 (3H, *d*, J = 3·5 Hz, C-21), 3·11 (1H, *m*, C-3α), 3·56 (1H, *m*, C-16), 4·36 (1H, *m*, C-22), 5·33 (1H, *m*, C-6). MS: *m/e* 418 (M^+), 400 (M^+-H_2O), 300 (400–100) base peak (*m** 225). H.R. MS: 418·3441 (M^+); calc. MW for $C_{27}H_{46}O_3$: 418·3447.

Calibagenin triacetate. Prepared by the usual method and purified by PLC. Crystals, mp 146–147°, R_f 0·48 on Si gel G with $CHCl_3$–MeOH–Me_2CO (9:1:1). (Found: C, 72·76; H, 9·58; O, 17·32. $C_{33}H_{52}O_6$ requires: C, 72·76; H, 9·62; O, 17·62). IR($CHCl_3$): 1730 (*s*), 1200–1245 (a 2 peak band) cm^{-1}. NMR ($CDCl_3$): δ 2·05 (9H, *s*, 3 Me-CO), 0·86 (6H, *d*, J = 5 Hz, C-26 and C-27), 0·91 (3H, *s*, C-18), 1·03 (3H, *s*, C-19), 1·27 (3H, *d*, J = 5 Hz, C-21), 4·58, 4·73, 4·83 (1H each, *m*, < CH-OAc). 5·28 (1H, *m*, C-6). MS: *m/e* 544 (M^+), 484 (M^+–AcOH), 424 (484–AcOH), 364 (424-AcOH), found metastable 177 (calc. 177·3), 253 (364–111) found metastable 176 (calc. 176·3), 171 (side chain $C_{10}H_{19}O_2^+$), 111 (side chain-AcOH), 60 (AcOH), 59 ($MeCOO^+$).

Calibagenin tri-m-brombenzoate. Prepared by the usual method, purified by column chromatography on Si gel G and crystallized from $CHCl_3$–Me_2CO, mp 112–114°. (Found: C, 59·33; H, 5·86; Br, 25·08. $C_{48}H_{55}O_6Br_3$ requires C, 59·57; H, 5·73; Br, 24·78). IR($CHCl_3$): 2940, 2860, 2485 (C-H), 1720, 1280, 1250, (RCOO), 1568 (aromatic ring) cm^{-1} MS: *m/e* 964 (M^+), 744 (M^+-BrC_6H_4COOH), 564 (764-BrC_6H_4COOH), 364 (564-BrC_6H_4COOH), 311 (whole side chain), 200 (*m*-Brombenzoic acid).

Dihydrocalibagenin (**2**). Calibagenin (150 mg) was hydrogenated in THF (75 ml) with PtO_2 (150 mg) at 24° and 583 mm Hg for 24 hr; 10·8 ml of H_2 were absorbed (calc. for one double bond 11·3 ml). Crystallization from MeOH gave needles, mp 186–187°. R_f on TLC II had the same R_f as (**1**), but gave a green color after spraying with 5N H_2SO_4; calibagenin gave a purple color. IR: it had no band at 1625 cm^{-1}. NMR($CDCl_3$): δ 0·785 (6H, *d*, J = 4Hz, C-26 and C-27), 0·855 (3H, *s*, C-18), 0·91 (3H, *s*, C-19), 0·965 (3H, *d*, J = Hz, C-21), no signal at δ 5·33.

Oppenauer oxidation of calibagenin. Calibagenin (**1**) was refluxed for 5 hr in toluene with aluminium isopropoxide and cyclohexanone to give a mixture of 5 substances. PLC permitted the isolation of 1 product which was crystallized from MeOH, m.p. 59–60°. IR ($CHCl_3$): 1642, 1375 cm^{-1}; UV ($CHCl_3$): 229, 236 nm (log ϵ = 4·7).

Cholest-4-ene-3,16,22 trione (**6**). Oppenauer oxidation of calibagenin (1g) for 1 hr reflux gave a mixture of products. By preparative TLC white needles were isolated, mp 76–77°. (Found: C, 77·92; H, 10·38; O, 11·87. $C_{27}H_{40}O_3$ requires C, 78·59; H, 9·77; O, 11·63). IR ($CHCl_3$): 2955, 2920 (C–H), 1736 (5-membered ring ketone), 1717 (aliphatic ketone), 1670 and 1618 (α,β-unsaturated ketone) cm^{-1}. UV ($CHCl_3$): λ max 245 nm (log ϵ 4·18).

Cholestane-3,16,22 trione (**3**). Dihydrocalibagenin (**2**) (730 mg) was dissolved in Me_2CO (150 ml) and maintained at 5–10° stirring with Jones' reagent [9] (8 ml) for 1 hr. The stirring was continued for 30 min and H_2O (100 ml) added. Crystals (500 mg) were obtained and recrystallized from MeOH to give needles (450 mg), m.p. 166–167°, R_f 0·85 on Si gel G with $CHCl_3$–Me_2CO (9:1:1). (Found: C, 78·23; H, 10·29; O, 11·45. $C_{27}H_{42}O_3$ requires C, 78·21; H, 10·21; O, 11·58). IR ($CHCl_3$): 2935–2900, 2840 (C–H), 1738 (α substituted 5-membered ring ketone), 1713 (6-membered ring ketone and aliphatic ketone), 1470, 1380 (Me and CH_2), 1420 (CH_2) cm^{-1}. NMR ($CDCl_3$): δ 0·86 (6H, *d*, J = 4 Hz, C-26 and C-27), 0·980 (3H, *s*, C-18), 1·08 (3H, *s*, C-19), 1·20 (3H, *d*, J = 10 Hz, C-21), 1·64 (8H, *m*, CH_2 groups in α position to CO), 2·66 (1H, *d*, J = 3·5 Hz, C-20). Ms: *m/e* 414 (M^+), 399 (M^+-CH_3), 358 (M^+-56 (McLafferty rearrangement in the side chain, 343 (358–15), 288 (M^+-126, McLafferty rearrangement of the carbonyl at C-16 with transfer of one H from C-21), 273, 271 (loss of side chain from 399 with transfer of 2H from the nucleus), 217 (loss of whole side chain plus C-15, C-16 and C-17), 126 (whole side chain), 43 (base peak).

Diketo-dicarboxylic acid (**4**). Dihydrocalibagenin (**2**, 1·6 g) was oxidised as previously described with Jones' reagent but with 1 excess (3 ml) of reagent added. The mixture was stirred for 4 hr; product showed 2 compounds (R_f 0·85 and 0·0) on TLC with $CHCl_3$–MeOH–Me_2CO (9:1:1) which were separated by PLC. The product with R_f 0·85 was identified as the triketone (**3**); the other crystallized from C_6H_6–hexane to give plates, mp 217–218°. (Found: C, 70·19; H, 9·21; O, 21·24. $C_{27}H_{42}O_6$ requires C, 70·10; H, 9·15; O, 20·75), neutralization equivalent 231 (calc. 231·2); IR ($CHCl_3$): broad band from 3525 to 2850 (characteristic for acids), 1738 (α substituted 5-membered ring ketone), 1713 and 1700 (carbonyl groups) cm^{-1}. NMR (DMSO-d_6): δ 0·74 (6H, *d*, J = 4Hz, C-26 and C-27), 0·82 (3H, *s*, C-18), 0·90 (3H, *s*, C-19), 1·0 (3H, *d*, J = 10Hz, C-21), 2·5 (1H, C-17), 2·7 (1H, C-20), 10·78 (2H, *s*, 2-COOH). MS: *m/e* 462 (M^+), 444 (M^+-18), 399 (444-COOH), 371 (399-CO), 358 (444-86) (loss of OC–O–CO–CH_2), Found metastable 288 (calc. 288·6). This fragmentation is possible only if the anhydride is asymmetric, therefore ring A is opened between C-3 and C-4 and not between C-2 and C-3. This explains why the fragment of *m/e* 358 loses one Me group by the elimination of C-19 to give the fragment at *m/e* 343, metastable 328·5 (calc. 328·6). Other MS ions at *m/e* 231 (358-side chain), metastable 149 (calc. 149·05), 217 (loss of side chain with 3C of ring D), 161 (217–56, loss of CH_2–CH=C=O), 127 (side chain), 99 (elimination of the side chain between C-20 and C-22), 57 [$(Me)_2C=CH_2$], 43 (base peak, MeCO).

Jones' oxidation for 6 hr gave only the diketodicarboxylic acid (**4**).

Cholestane (**5**) Hydrazine hydrate (0·2 ml) and HOAc (3 drops) were added to cholestanetrione (**3**) (157 mg) in MeOH (3 ml). The soln was boiled for 30 min and crystallized to give a light yellow hydrazone, which was heated with KOH (500 mg) at 90° for 1 hr [10] until no more N_2 was evolved. The product (80 mg) was filtered on neutral Al_2O_3 (2g) in C_6H_6 and plates (40 mg) were obtained, mp 78–79° identical to cholestane by mmp, IR and (R_f) TLC with C_6H_6–AcOEt (75:25). (Found: C, 87·37; H, 12·85. Calc. for $C_{27}H_{48}$: C, 87·02; H, 12·98).

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