Lupeol. mp 215°; IR ν_{max}^{KBr} cm^{-1}: 3340(OH), 1640, 875 (=CH_2); NMR ($CDCl_3$, TMS); δ0.76, 0.79, 0.84, 0.96 and 1.02 (six methyl singlets), 1.66 (vinylic methyl, 3H, *s*), 3.16 (carbinol hydrogen, 1H, *d*, J = 6 Hz), 4.54 and 4.66 (=CH_2, 1H each, *d*, J = 1.5 Hz). Acetylation formed a monoacetate, mp 205°; IR ν_{max}^{KBr} cm^{-1}: 1735, 1250 (ester). NMR ($CDCl_3$, TMS): δ 2.00 (acetyl, 3H, *s*), C-3 proton shifted to δ4.4 ppm (1H, *d*, J = 5.5 Hz). MS *m/e*: 426 (M^+), base peak at 218, other peaks at 220, 189 and 207 [6]. Jones oxidation yielded lupen-3-one, mp 169–170°. Reduction of lupen-3-one with $NaBH_4$ gave mainly lupeol and a small amount of epilupeol. Comparison with an authentic sample confirmed the product as lupeol.

Lup-(20)29-ene-2α,3β-diol. mp 234°; $C_{30}H_{50}O_2$ M^+ 442; IR ν_{max}^{KBr} cm^{-1}: 3340(OH), 1380(gem dimethyl), 880, 1640(=CH_2); NMR ($CDCl_3$, TMS): δ0.79, 0.88, 0.93, 0.98, 1.02 (tertiary methyl singlets), 1.68 (3H, *s*, vinylic methyl), 3.2 (1H, *dd*, J = 10 Hz, 11 Hz) and 3.55 (1H, *m*, $W_{\frac{1}{2}}$ 20 Hz) (3αH and 2βH respectively), 4.55 and 4.70 (1H each, *d*, J = 1.5 Hz, exo = CH_2). MS showed diagnostically important peaks for lupeol derivatives at *m/e* 442 (M^+), 427 (M–Me), 424 (M-18), 236, 223, 218, 189, 203, 218, 205 [2]. Acetylation yielded a diacetate, mp 131°; $C_{34}H_{54}O_4$, M^+ 526; IR ν_{max}^{KBr} cm^{-1}: 1740 (ester), 885, 1640 (=CH_2); NMR ($CDCl_3$, TMS): δ 1.92 and 2.01 (3H each, *s*, acetyl). Jones oxidation yielded a diosphenol, UV λ_{max} 273 nm (ε 7800); IR ν_{max}^{KBr} cm^{-1}: 885, 1650 (=CH_2), 1385 (gem dimethyl), 3450 (OH); NMR ($CDCl_3$, TMS) δ0.78, 0.82, 0.95, 1.01, 1.20 (six tertiary Me, *s*), 1.68 (3H, *s*, vinylic Me), 4.50 and 4.65 (=CH_2), 6.29 (1H, *s*, vinylic proton, C-1).

Olean-(13)18-ene-2β,3β-diol. The new oleanenediol (160 mg), crystallized from EtOH, mp 228° (Found C, 80.95; H, 11.05. $C_{30}H_{50}O_2$ requires: C, 81.40; H, 11.40%). IR ν_{max}^{KBr} cm^{-1}: 1385 (gem dimethyl), 3450 (OH). NMR and MS described in the results. A diacetate was formed upon acetylation, mp 114° (Found: C, 77.95; H, 10.27. $C_{34}H_{54}O_4$ requires: C, 77.50; H, 10.30%) IR ν_{max}^{KBr} cm^{-1} 1385 (gem dimethyl), 1725, 1250 (ester); NMR ($CDCl_3$, TMS); δ0.8, 0.86, 0.98, 1.10 and 1.20 (8 tertiary Me, *s*), 1.94 and 2.02 (3H, each, *s*, 2 × OCOMe), 4.50 (1H, *d*, J = 6.5 Hz, 3αH), 5.20 (1H, *d*, J = 2.5 Hz, 2αH).

Olean-(13)18-ene-2β,3β-acetonide (3). 30 mg of the diol dissolved in 1 ml dry Me_2CO was added to 200 mg *p*-toluensulfonic acid in Me_2CO and kept at room temp. for 1 hr. Purification on a small column of Si gel gave the amorphous acetonide (Found: C, 81.95; H, 11.35. $C_{33}H_{54}O_2$ requires: C, 82.10; H, 11.20%). IR ν_{max}^{KBr} cm^{-1} 858, 1050, 110, 1158 (acetonide); NMR ($CDCl_3$, TMS): δ0.78, 0.82, 0.94, 1.00, 1.11, 1.20 (eight tertiary Me, *s*), 1.38 and 1.42 (3H each, *s*, methyls of acetonide), 3.52 (1H, *d*, J = 6.5 Hz, 3αH) and 4.2 (1H, *d*, J = 2.5 Hz, 2αH).

Diosphenol. 50 mg of the diol dissolved in 5 ml Me_2CO was treated with CrO_3–AcOH (25 mg of CrO_3 in 1 ml AcOH) at room temp. for 5 min, then diluted with H_2O, extracted with Et_2O and the residue purified by PLC (Si gel G, $CHCl_3$–C_6H_6), mp 263°, +$FeCl_3$ reaction, UV λ_{max}^{EtOH} nm: 275 (ε 7600) and 256 (ε 12000); IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1380 (gem dimethyl), 1725, 1630 (carbonyl bands); NMR ($CDCl_3$, TMS): δ0.79, 0.84, 0.96, 1.05, 1.12, 1.24 (eight tertiary Me, *s*), 6.33 (1H, *s*, C-1).

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REFERENCES

1. Ulubelen, A. and Brieskorn, C. H. (1975) *Phytochemistry* **14**, 820.
2. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II. p. 128. Holden-Day Inc.
3. Williams, D. H. and Bhacca, N. S. (1965) *J. Am. Chem. Soc.* **86**, 2742.
4. Kumar, N. and Seshadri, T. R. (1976) *Phytochemistry* **15**, 1417.
5. Kumar, N. and Seshadri, T. R. (1975) *ibid* **14**, 521.
6. Herz, W., Santhanam, P. S. and Wahlberg, I. (1972) *ibid* **11**, 3062.

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STRUCTURE OF BAROGENIN FROM *SOLANUM TUBEROSUM*

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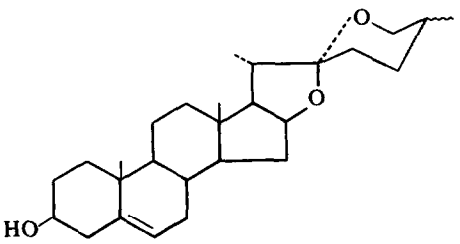
Key Word Index—*Solanum tuberosum*; Solanaceae; steroidal sapogenin; kryptogenin; barogenin; spirostan biosynthesis.

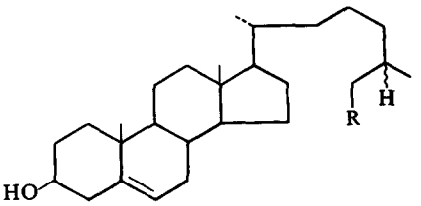
Abstract—The budding tuber of *Solanum tuberosum* accumulated barogenin. Its structure was determined by chemical and spectroscopic studies as (25*S*)-3β,26-dihydroxy-cholest-5-ene-16,22-dione, the (25*S*)-epimer of kryptogenin. The biogenetic relationship between barogenin and spirostanols is discussed.

INTRODUCTION

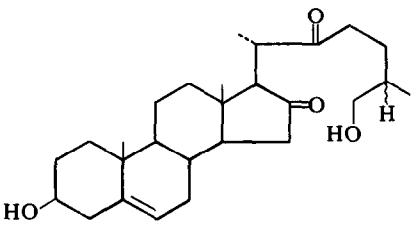
The biogenesis of spirostanols, for example, diosgenin (**1a**) and yamogenin (**1b**), is presently under investigation in several laboratories [1–7]. Most of the hypothesis, based on the results of feeding experiments, suggest that cholesterol (**2a**) is oxygenated at C-26 (**2b**), C-16, and C-22, in that order, and then enzymatically converted to a spirostan. However, there is no report on the presence of these compounds, and the presence of 26-hydroxycholesterol (**2b**) or its derivatives which carry an oxygen function at C-16 or C-22 has not been recorded in plants which contain spirostanol. Therefore it seemed significant to



1a: (25*R*) Diosgenin
1b: (25*S*) Yamogenin



2a: Cholesterol, R = H
2b: 26-Hydroxycholesterol, R = OH



3a: (25*R*) kryptogenin
3b: (25*S*) Barogenin

examine the seasonal variation of steroidal components in plants and then to isolate possible intermediates. We found a new compound (**3b**) in the bud and the budding tuber of *Solanum tuberosum* [Japanese variety name, Danshaku (Baron)], which increased with the growth of the tuber and reached a maximum concentration at the budding period. We now report the isolation and the structure of elucidation of this compound (**3b**) which is the (25*S*)-epimer of kryptogenin.

RESULTS AND DISCUSSION

Analysis of the neutral fraction from *S. tuberosum* by TLC showed that **3b** was absent from the aerial portion but appeared and increased in the tuber with its growth. This compound reached a maximum concentration at the budding period. Extraction of the dried material of bud and epidermis of budding tubers gave a glycoside mixture. Hydrolysis of the glycoside afforded steroidal alkaloids and neutral compounds. Purification of the neutral fraction by silica gel column chromatography and PLC yielded yamogenin (**1b**) and **3b**, mp 196–199°, $[\alpha]_D$ − 88.8°. From the elemental analysis and MS (M^+-H_2O at *m/e* 412), the molecular formula of **3b** was established as $C_{27}H_{42}O_4$. Its IR spectrum indicated the presence of a hydroxyl (3400–3500 cm^{-1}), a five-membered ring carbonyl (1730 cm^{-1}) and a carbonyl group (1705 cm^{-1}). The NMR spectrum of **3b** showed signals for two tertiary methyl groups at δ 0.82 (18-Me) and 1.05 (19-Me), two secondary methyl groups at 0.96 (*d*, J = 6 Hz) and 1.06 (*d*, J = 7 Hz), one hydroxymethyl group at 3.48 (*d*, J = 6 Hz), one hydroxy methine at 3.50 (*m*) and one olefinic proton at 5.37 (1H, *m*, 5-H).

These results indicate that **3b** has a cholestene skeleton with two hydroxyl groups and two carbonyl groups. Treatment of **3b** with acetic anhydride in pyridine gave its diacetate mp 136–137.5°. The NMR spectrum showed two new acetoxy methyl signals at δ 2.03 (*s*) and 2.05 (*s*) while the methylene and methine signals shifted to 3.94 and 4.60, respectively. The methylene and methine signals are assignable to the C-26 proton and 3α-proton, respectively, by comparison with the NMR spectrum of 26-hydroxycholesterol (**2b**) [8]. From the biogenetic analogy to yamogenin (**1b**), it is deduced that two carbonyl groups are located at C-16 and C-22. The spectral properties of **3b** were essentially identical with those of kryptogenin (**3a**) which was isolated from *Dioscorea* plants.

However, from the difference of melting point (kryptogenin; 189°) and the fact that *S. tuberosum* contains steroidal compounds of (25*S*)-configuration, **3b** was assumed to be a C-25 epimer of kryptogenin. Reduction of **3b** with $NaBH_4$ gave yamogenin (**1b**), mp 199–201°, as a major product. The identity of the product with yamogenin (25*S*) was confirmed by IR, TLC, GLC, MS, and mmp with authentic yamogenin; no trace of diosgenin (25*R*) (reported mp 204–207°) was detected in the products. From these results, **3b** was concluded to be (25*S*)-3β,26-dihydroxy-cholest-5-ene-16,22-dione, and named barogenin after the Japanese variety name of Danshaku (Baron) for *S. tuberosum*.

Marker and Lopez [9] found that Beth root yielded increasing amounts of diosgenin (**1a**) from decreasing amounts of kryptogenin (**3a**) during storage. Similarly, from TLC evidence barogenin (**3b**) appeared only at the growing and budding period of the tuber, and increased in parallel with the increment of yamogenin (**1b**). From these facts, it is likely that barogenin is a precursor in the biosynthesis of yamogenin. Although Heftmann and Bennet [1] reported that kryptogenin (**3a**) was not incorporated into diosgenin (**1a**), the experimental method used may have been unsuitable.

EXPERIMENTAL

Mps are uncorrected. Optical rotations were measured in $CHCl_3$ soln; NMR spectra were determined in $CDCl_3$ with TMS as an internal standard; IR spectra were measured in $CHCl_3$

soln. TLC was performed on Si gel HF_{254} (Merck, Type 60), and Si gel (Merck, 70–230 mesh) was used for column chromatography.

Isolation of the aglycone mixture. The tuber of *Solanum tuberosum* L. was harvested in early September 1975. At this time, they were separated into 3 groups according to diameter (*ca* 2, 5, and 8 cm). Treatment of epidermis of each tuber by the procedure described below gave a neutral compound. The remainder of the tubers were allowed to stand at room temp. for about 4 months in a dark room and the epidermis of just budding tuber was treated in the same manner. By the use of TLC, 4 samples were compared (colour producing reagent: $SbCl_3$). For extraction, the epidermis of just budding tuber and its bud were used. The dried material (2 kg) was extracted with MeOH by a Soxhlet apparatus until the extract became colourless. Evaporation of the solvent gave a brownish crude glycoside (97 g). It was hydrolyzed by refluxing in 2 l. of 1N HCl in MeOH for 6 hr, the hydrolysate was made alkaline with 5N NaOH and then extracted 3 × 400 ml portions of Et_2O. The alkaloidal fraction was removed with 5% tartaric acid solution (0.4 l × 3). The Et_2O phase was dried over Na_2SO_4 and evaporated to yield 6.9 g of neutral fraction.

Barogenin (3b). The neutral fraction was chromatographed over 210 g of Si gel and the column was eluted successively with C_6H_6, C_6H_6–Et_2O (19:1, 9:1, and 4:1), $CHCl_3$, and MeOH. The eluate with $CHCl_3$ and MeOH gave 80 mg of barogenin fraction. PLC (Si gel HF_{254}, 10% MeOH in $CHCl_3$) of the barogenin fraction and two recrystallizations of crude barogenin from Me_2CO gave needles (19 mg), mp 196–199°, $[\alpha]_D^{18}$ − 88.8° (*c*, 0.36 in $CHCl_3$). MS *m/e*: 412 (M^+-H_2O), 397 (M^+-H_2O-Me), 382 (M^+-H_2O-2Me), 115 (base peak). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400–3500, 1730, 1705. NMR (δ): 0.82 (3H, *s*, c-18), 0.96 (3H, *d*, *J* = 7 Hz, c-27), 1.05 (3H, *s*, c-19), 1.06 (3H, *d*, *J* = 7 Hz, c-21), 3.48 (2H, *d*, *J* = 6 Hz, c-26), 3.50 (1H, *m*, 3α-H), 5.37 (1H, *m*, c-6). (Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.24; H, 9.75%) Barogenin (18 mg) was acetylated in the usual manner and recrystallization of the product from MeOH gave 14 mg of barogenin acetate (**3b**-acetate), mp 136–137.5°. MS *m/e*: 454 (M^+-AcOH), 394 (M^+-2AcOH), 115 (base peak). NMR (δ): 0.83 (3H, *s*, c-18), 0.96 (3H, *d*, *J* = 7 Hz, c-27), 1.07 (3H, *s*, c-19), 1.07 (3H, *d*, *J* = 7 Hz, c-21), 2.03 (3H, *s*, O-Ac), 2.05 (3H, *s*, O-Ac), 3.94 (2H, *d*, *J* = 6 Hz, c-26), 4.60 (1H, *m*, 3α-H), 5.38 (1H, *m*, c-6). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 (shoulder), 1730, 1705, 1260.

Conversion of barogenin acetate (3b-acetate) into yamogenin (**1b**). A mixture of 10 mg barogenin acetate, 5 ml MeOH, and 20 mg $NaBH_4$ was stirred at room temp. for 2 hr and the excess $NaBH_4$ was destroyed by dilute HCl. The mixture was extracted with $CHCl_3$ and worked up as usual. The solvent was evaporated to dryness and the residue was recrystallized from Me_2CO to give 3 mg of compound **1b**, mp 199–201°, MS *m/e*: 414 (M^+), 399, 396, 384, 139 (base peak). The identity of this compound with yamogenin (25*S*) was confirmed by IR, TLC [solvent system: EtOAc–cyclohexane (1:1)], GLC (column: 1.5% SE-30, column temp. 250°), MS, and mmp with authentic yamogenin (mp 200–201°, mmp 197–200°). IR ν_{max}^{Nujol} cm^{-1}: 3450, 1663, 1220, 1066, 996, 923, 900, 860.

REFERENCES

1. Bennet, R. D. and Heftmann, E. (1966) *Phytochemistry* **4**, 577.
2. Bennet, R. D., Heftman, E. and Joly, R. A. (1970) *Phytochemistry* **9**, 349.
3. Heftmann, E. and Weaver, L. M. (1974) *Phytochemistry* **13**, 1801.
4. Tschesche, R. (1972) *Proc. Roy. Soc. (London)* B **180**, 187.
5. Tschesche, R. and Piestert, G. (1975) *Phytochemistry* **14**, 435.
6. Canonica, L., Ronchetti, F. and Russo, G. (1972) *Phytochemistry* **11**, 243.
7. Tomita, Y. and Uomori, A. (1971) *Chem. Commun.* 284.
8. Schubert, K., Kaufmann, G., and Budzikiewicz, H. (1969) *Biochim. Biophys. Acta* **176**, 170.
9. Marker, R. E. and Lopez, J. (1947) *J. Am. Chem. Soc.* **69**, 2386.

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NEW ARBUTIN DERIVATIVES FROM LEAVES OF *GREVILLEA ROBUSTA* AND *HAKEA SALIGNA*

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Key Word Index—*Grevillea robusta*; *Hakea saligna*; Proteaceae; arbutin; 2-*p*-coumaroylarbutin; 6-*p*-coumaryl-arbutin; 6-caffeylarbutin; 6-*p*-hydroxybenzoylarbutin.

Previous work has shown the presence of 5 *n*-alkyl resorcinols [1], mono and bis norstriatols [2] in the wood and rutin, 2,5-dihydroxycinnamic acid, methyl 4-hydroxycinnamate, robustol and related macrocyclic phenolics [3] and arbutin in the leaves of *Grevillea robusta*. We felt that methyl 4-hydroxycinnamate might be an artifact produced by methanolysis during extraction and hence reinvestigated the leaves. From the acetone extracts, by extensive column and preparative TLC methods, arbutin and two new arbutin derivatives were separated. By the usual technique of permethylation studies and NMR analysis of their acetates, the last two were identified as 2-*p*-coumaryl- and 6-*p*-coumaryl-arbutins. Of these the former was in major amount. *p*-Hydroxybenzoyl-arbutin was absent.

The leaves of *Hakea saligna* were similarly examined and the combined acetone and methanol extracts yielded 2-*p*-coumaryl- and 6-caffeyl- and 6-*p*-hydroxybenzoyl-arbutins. The last was the major glycoside. No 6-*p*-coumarylarbutin was detected in the leaves. A comparison of the arbutin derivatives in the leaves of the two Proteaceae shows that *p*-hydroxybenzoylarbutin is restricted to *H. saligna* and 6-*p*-coumarylarbutin to *G. robusta*. Such differences may have taxonomic implications. Previously known arbutin derivatives from other sources are 2-*O*-gallyl- and 6-*O*-gallylarbutins, *p*-gallyl-arbutin (4) and 2-*O*-caffeylarbutin [5].

During a study of the NMR spectra of the acetates of the new glycosides, it was observed that all the protons of the cinnamyl moiety showed considerable upfield