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TRITERPENOIDS OF *COLLETIA SPINOSISSIMA*

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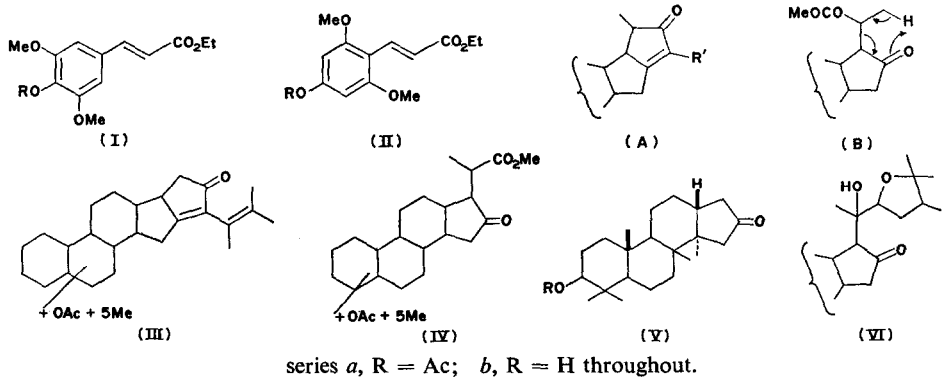
Key Word Index—*Colletia spinosissima*; Rhamnaceae; triterpenoids; lupenone; lupeol; daucosterine; two new sapogenins.

Abstract—The neutral components from the stems of *Colletia spinosissima* Gmel. include lupenone, sitosterol lupeol, and daucosterine. Acid hydrolysis of the glycoside fraction afforded ethyl sinapate and two homologous degradation products from the corresponding sapogenins. Mild hydrolysis of the glycoside fraction afforded the intact sapogenins, which have been tentatively assigned the structures (VIIb) and (VIIIb). The chemistry of the latter compounds has been explored.

INTRODUCTION

AS PART of a survey of Chilean flora,† *Colletia spinosissima* Gmel. (Rhamnaceae) has been investigated. Comin *et al.*[1] previously isolated magnocuranine and a new alkaloid, called colletine, from Argentinian samples of this species. We have confirmed these findings and, furthermore, have also studied the neutral fraction obtained from dried stems of the plant. In this way initial extraction afford hentriacontane, lupenone, sitosterol, and lupeol. The ethanolic fraction afforded a glycosidic fraction and daucosterine. Mild acid hydrolysis of the glycoside fraction afforded three new compounds, shown to be ethyl sinapate and two new homologous sapogenins. On the basis of the following chemical and spectral evidence these were assigned the structures (Ib), (VIIb), and (VIIIb) respectively.



series *a*, R = Ac; *b*, R = H throughout.

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† For the previous paper in this series see SILVA, M., MUNDACA, J. M. and SAMMES, P. G. (1971) *Phytochem.* **10**, 1942.

[1] SANCHEZ, E. and COMIN, J. (1967) *Tetrahedron* **23**, 1139.

RESULTS

Initial hydrolysis of the glycosidic fraction (see Experimental) with 2·5 N HCl, followed by chromatography on alumina, afforded three fractions. One of these was a low melting phenol, initially characterized as its acetyl derivative. Elemental analysis gave its formula as $C_{15}H_{18}O_6$, which was confirmed by its MS. NMR showed the acetyl derivative to contain two methoxyl groups (as a singlet), one acetyl function, an ethyl ester group, and four remaining protons, two of which were present as an *AB* pair, *J* 16 Hz, characteristic in chemical shifts for a conjugated ester of the *trans*-cinnamic acid type. The two remaining protons occurred as a singlet in the aromatic region. The coincidence of the aromatic and of the methoxyl protons was maintained in deuteriobenzene solution. Only two possible structures can accommodate the symmetry associated with these spectra, viz. (Ia) or (IIa). Calculation of the chemical shifts[2] favoured the former structure. Treatment of the cinnamate with either acid or base followed by acid and then irradiation did not afford a coumarin, as anticipated for a cinnamate flanked by *ortho* groups.[3] Instead acid afforded a phenol, m.p. 80–81° (from EtOH), identical to the crude ester isolated before acetylation and assigned as ethyl sinapate.[4] The product from base hydrolysis was identical in its properties to those recorded for sinapic acid. The ester (Ib) is possibly formed by *trans*-esterification during the ethanolic extraction procedure.

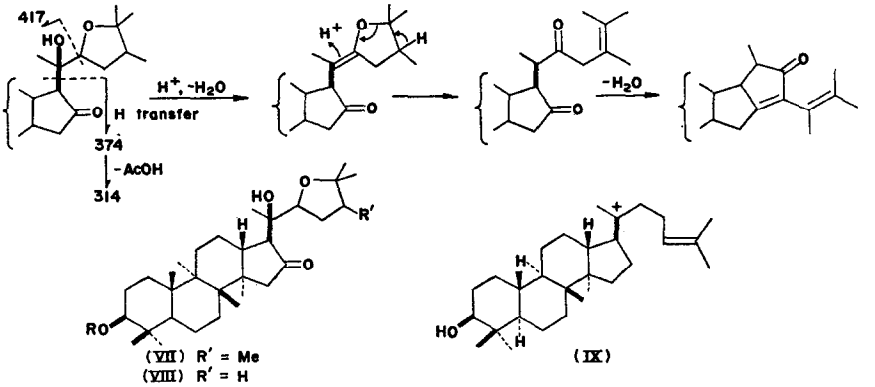
The second component from the aglycone fraction was shown to be a mixture of two homologous compounds by MS. These were not readily separable, but acetylation afforded the acetate of the major component, which analysed as $C_{33}H_{50}O_3$, λ_{max} 224 nm (ϵ 14 400), ν_{max} 1736 (acetate), 1690 (unsaturated ketone), and 1620 cm^{-1} (C=C). This compound formed a 2,4-dinitrophenylhydrazone which confirmed the presence of the ketone function and accounts for all the oxygen atoms present. Its MS showed no strong, characteristic peaks other than for loss of acetic acid and methyl groups. A fairly strong peak at *m/e* 69 analysed as C_5H_9. Oxidation of the acetate afforded an acid which was methylated with diazomethane. This oxidation product was a cyclopentanone since the carbonyl absorption occurred at 1730 cm^{-1}. The formation of the cyclopentanone from the starting unsaturated ketone is consistent with the latter having part structure (A), producing the ketone (B) by oxidation and methylation. The part structure (B) is also consistent with its MS fragmentation pattern which shows strong peaks at *m/e* 374 and 373, corresponding to the McLafferty cleavage (see B) and to loss of the carbomethoxy group and carbon monoxide respectively. The oxidation of the cyclopentenone is reminiscent of the oxidative cleavage of the cyclopentenones obtained from sapogenoic acids.[5] In order to accommodate the remaining spectral information the rest of the molecule is formulated as a saturated tetracyclic nucleus of the steroid type. Thus a part structure for the cyclopentenone is (IIIb) and for the derived ester is (IVb).

The third compound isolated from the initial acid hydrolysate was a cyclopentanone, $C_{24}H_{38}O_3$ (as acetate). The NMR spectrum showed no vinylic protons and the solid gave a negative tetranitromethane test. Thus this keto acetate corresponds to a tetracyclic system. The NMR spectrum also showed the presence of five methyl singlets. Thus this compound is an androstane derivative. The acetate function showed an adjacent proton NMR

[2] BALLANTINE, J. A. and PILLINGER, C. T. (1967) *Tetrahedron* **23**, 1691.
[3] DEAN, F. M. (1963) *Naturally Occurring Oxygen Ring Compounds*, p. 179, Butterworths, London; EDWARDS, K. G. and STOKER, J. R. (1968) *Phytochem.* **7**, 73.
[4] *Dictionary of Organic Compounds* (1965) (edited by G. HARRIS), p. 1672, Eyre & Spottiswoode, London.
[5] MARKER, R. E., SHABICA, A. C. and TURNER, D. L. (1941) *J. Am. Chem. Soc.* **63**, 2274; BARTON, D. H. R., KULKARNI, Y. D. and SAMMES, P. G. (1971) *J. Chem. Soc.* **C**, 1149.

resonance characteristic of a 3β-substituent flanked by only two vicinal protons, viz. as a 3β-acetate with two methyl groups at position 4. That the carbonyl group was a cyclopentanone was indicated by its IR absorption, ν_{max} 1735 cm^{-1}. A tentative structure for this compound is the ketone (Va). That the ketone function was at position 16 was indicated by a negative Zimmerman test.[6] Europium shift experiments were consistent with the assigned structure (Vb) and especially for the presence of the two methyl groups at position 4.[7]

When the glycoside fraction was hydrolysed with acid under less vigorous conditions, using N HCl, very little of the compounds (IIIb) or (Vb) were isolated. Instead, the major steroidal component was a mixture of two homologous compounds, analysed as the derived monoacetates as $C_{33}H_{54}O_5$ (major component) and $C_{32}H_{52}O_5$. Treatment of the major component with 1N NaOH immediately produced the alcohol of the cyclopentanone (Vb), whilst treatment of the major product with 2·5 N HCl produced the alcohol corresponding to the cyclopentenone (IIIb). That the cyclopentanone (Vb) and the cyclopentenone (IIIb) are derived from a common precursor implies that the latter has the part structure (VI). The steroidal structure of the sapogenin can therefore be presented as (VIIb). A simple reversed aldol reaction with base (e.g. on alumina) accounts for the formation of the cyclopentanone, whilst acid must induce dehydration followed by an acid catalysed condensation between positions 16 and 23, as depicted in Scheme 1. The methyl substitution pattern of the nucleus follows from biogenetic considerations. The co-occurrence of the lower homologue, a triterpene, is, of course, not unusual; this has been assigned structure (VIII). The stereochemistry about position 22 (and 24) remains undefined; that at position 20 is assumed only on the basis of considerable precedent.*



The new sterols (VIIb) and (VIIIb) can be considered as members of the relatively rare group of dammarane steroids. Since lupeol and lupenone were also isolated from this plant the fusidane skeleton can be ruled out. The co-formation of both lupane and dammarane derivatives in a member of the Rhamnaceae has precedence in the co-production of ebelin lactone and betulic acid by *Emmenospermum alphitonoiides* F. Muell.[8] As in that case, the production of lupeol, lupenone and the sterols (VIIb) and (VIIIb) can be envisaged as arising via the common biogenetic precursor (IX).[9]

* It is stressed that the structures assigned to the triterpenes (VIIb) and (VIIIb) are as yet tentative and a correlation with compounds with defined configuration remains to be done.

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EXPERIMENTAL

M.ps were determined on a Kofler block and are uncorrected. The NMR spectra were determined in $CDCl_3$ containing tetramethylsilane as internal reference. The MS were determined with an AEI MS9 double focusing instrument. Merck SiO_2 and Al_2O_3 were used for chromatography. Light petrol. refers to the fraction, b.p. 60–80°.

Extraction. 18·7 kg of dried stems from *Colletia spinosissima* were extracted in a Soxhlet to exhaustion with C_6H_6 then EtOH. The former afforded 335 g and the latter 1·83 kg of crude extracts.

Benzene fraction. This extract (6 g) was chromatographed on alumina (grade III, 60 g) to yield the following components: *Hentriacontane* (90 mg). Eluted with light petrol. m.p. 55–57°; ν_{max}^{Nujol} 1473, 735, 724 cm^{-1} MS bands at 436, etc. consistent with this structure. The fraction was contaminated with lower and higher homologues of saturated hydrocarbons. *Lupenone* (300 mg). Eluted with light petrol. m.p. 163–165°, $[\alpha]_D^{21}$ +57·4° (*c* 1·0, $CHCl_3$) (lit.[10] m.p. 165° $[\alpha]_D$ +57·6°), ν_{max}^{Nujol} 1705 cm^{-1}, identical NMR and MS patterns to an authentic sample, as well as identical m.p., m.m.p. and co-chromatography on TLC. The material also gave an identical 2,4-dinitrophenylhydrazone to that reported in the literature (m.p. 214–216°, lit,[10] m.p. 214°). *Sitosterol* (120 mg). Eluted with C_6H_6–light petrol. (1:1) m.p. 135° (from EtOH), λ_{max}^{EtOH} 206 nm (ϵ 5000) (end absorption), ν_{max}^{Nujol} 3425, 1460, 1376, 1051 cm^{-1}. This compound was identical to an authentic sample by m.p., m.m.p. TLC and NMR. Acetylation with Ac_2O–pyridine afforded the acetate, m.p. 120–122°, undepressed by m.m.p. with an authentic sample. *Lupeol* (390 mg). Eluted with C_6H_6, m.p. 195–215° (from EtOH), and not improved by further recrystallization. NMR analysis showed this to be almost pure lupeol (by direct comparison). Acetylation afforded the acetate, m.p. 215–219° (from EtOH) (lit.[11] m.p. 220°). Oxidation with Jones' reagent gave lupenone, identical to the sample isolated above and with an identical MS fragmentation pattern.[12]

EtOH extract. The concentrated extract slowly deposited a solid. Isolation of this by filtration afforded a crude sample of daucosterine. Recrystallization from EtOH afforded pure daucosterine (0·25 g), m.p. 275–283° (lit[13] m.p. 272–275°), ν_{max} 3450, 1445, 840, 800 cm^{-1}, identical to an authentic sample by TLC co-chromatography. Acetylation afforded the actate, m.p. 165–168° (from EtOH) (lit.[13] m.p. 167–168°), undepressed on m.m.p. with an authentic sample. Acid hydrolysis afforded glucose (identified by PC) and sitosterol. A portion (425 g) of the EtOH extract was hydrolysed with 2·5 N HCl at reflux for 7 hr. The solution was poured into cold H_2O and the precipitate was washed with H_2O, dried, and re-extracted with light petrol.–C_6H_6 (1:1) followed by C_6H_6. The two extracts were separately chromatographed through alumina (grade III) to afford a total of 3 compounds, described in order of elution.

Compound (1a). This material could not be isolated free. The product obtained by elution with C_6H_6–light petrol. (0·25 g) was acetylated at room temp. for 16 hr. The crude product crystallized, m.p. 124–127° (from light petrol.–Et_2O), λ_{max}^{EtOH} 224, 292 nm (20 300, 18 400), ν_{max}^{Nujol} 1740, 1710, 1603, 1460, and 1277 cm^{-1}, τ ($CDCL_3$) 2·4 (1H, *d*, *J* 16 Hz), 3·32 (2H, *s*), 3·7 (1H, *d*, *J* 16 Hz), 5·81 (2H, *q*, *J* 6·5 Hz), 6·23 (6H, *s*), 7·74 (3H, *s*), 8·72 (3H, *t*, *J* 6·5 Hz), τ (C_6D_6) 2·19 (1H, *d*, *J* 16 Hz), 3·48 (2H, *s*), 3·48 (1H, *d*, *J* 16 Hz), 5·80 (2H, *q*, *J* 6·5 Hz), 6·78 (6H, *s*), 8·00 (3H, *s*), 8·92 (3H, *t*, *J* 6·5 Hz), *m/e* 294 (M^+) (5%), 252(100), 249(5), 237(2), 224(6), 207(35), 180(30). (Found: C, 60·85; H, 6·30. $C_{15}H_{18}O_6$ requires: C, 61·24; H, 6·16%.) A sample of the acetate was hydrolysed with 3N HCl–dioxane (1:1) at reflux for 30 min. The product was purified from TLC (light petrol.–EtOAc, 1:3) to give needles, m.p. 80–81° (EtOH), λ_{max}^{EtOH} 240 (18 000), 330 nm (19 800), τ 2·46 (1H, *d*, *J* 16 Hz), 3·30 (2H, *s*), 3·77 (1H, *d*, *J* 16 Hz), 5·80 (2H, *q*, *J* 6·5 Hz), 6·17 (6H, *s*), 8·70 (3H, *t*, *J* 6·5 Hz), *m/e* 252 (M^+). Lit.[14] m.p. for ethyl sinapate, 80–81°. A sample of the ester acetate was hydrolysed with 1 N KOH for 20 min at reflux to give, after reacidification, the acid, m.p. 195–200° (from EtOH) (lit.[14] m.p. 192°).

Compound (III). Isolated by elution with light petrol. and crystallized from EtOH to give 350 mg, m.p. 242–244°. It gave a 2,4-dinitrophenylhydrazone, m.p. 215–220°, λ_{max} 387 nm. NMR showed this fraction to be a mixture of two homologous compounds. A sample was acetylated followed by purification by TLC (multiple elution) to give the *monoacetate* (III), m.p. 244°, $[\alpha]_D$ −11·6 (*c* 0·5, $CHCl_3$) λ_{max}^{EtOH} 224 nm (ϵ 14 700), ν_{max}^{Nujol} 1730, 1692, 1631, and 1233 cm^{-1}, τ 5·60 (1H, *dd*, *J* 6·8 Hz), 7·7 (2H, *m*), 8·00 (3H, *s*), 8·3 (6H, vinylic methyls), 8·55 (3H, vinylic methyl), 8·85 (3H, *d*, *J* 6 Hz, secondary methyl group), 8·95 (3H, *s*), 9·2 (9H, *s*) (the sample before TLC also showed a small peak at 2·80 due to the vinylic proton in the minor component); *m/e* 494 (M^+), 479, 434. (Found: 494·3770. $C_{33}H_{50}O_3$ Requires: 494·3760.) (Found: C, 80·3; H, 10·2. Required: C, 80·2; H, 10·2%.) Oxidation of the acetate (III) (10 mg) with CrO_3 (2 mg) in HOAc (10 ml) at room temp. for 12 hr afforded an acid, m.p. 283–300°. Esterification with an excess of CH_2N_2 in Et_2O gave the *methyl ester* (IV) m.p. 138–140°, $[\alpha]_D$ −12·5° (*c* 0·4, $CHCl_3$) ν_{max} 1740, 1240 cm^{-1}, *m/e* 460 (M^+), 445,

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[12] BUDZIKIEWICZ, H., WILSON, J. M. and DJERASSI, C. (1963) *J. Amer. Chem. Soc.* **85**, 3688.
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439 ($M^+ - MeO$), 400 ($M^+ - AcOH$), 385,374, 373 (base peak), 189. (Found: 460·3194. $C_{28}H_{44}O_5$ Requires: 460·3189.) (Found: C, 74·75; H, 10·1. $C_{28}H_{44}O_5$ Requires: C, 74·9; H, 9·7%).

Ketone (V). Eluted with C_6H_6 to give needles (230 mg), as alcohol, m.p. 215–217°, $[\alpha]_D$ −106·5° (*c* 1·03, $CHCl_3$), no λ_{max}, ν_{max}^{Nujol} 3490, 1735 cm^{-1}, τ 6·90 (1H, *m*), 7·8–8·1 (2H, *m*, spreading into the methylene envelope), 9·06 (Me, *s*), 9·11 (Me, *s*), 9·14 (Me, *s*), 9·21 (Me, *s*), 9·30 (Me, *s*). Oxidation with Jones' reagent gave a diketone, m.p. 122–126° (from EtOH), which gave a 2,4-dinitrophenylhydrazone, m.p. 218–225°. Acetylation of the hydroxy ketone gave the *acetate* (Va), m.p. 203–205° (from MeOH), $[\alpha]_D^{25}$ −49·2° (*c* 1·14, $CHCl_3$), no λ_{max}, ν_{max}^{KBr} 1735, 1235 cm^{-1}, τ 5·55 (1H, *m*), 8·00 (MeCO), 8·9–9·1 (5 × Me, *s*); *m/e* 374, 314, 299, 271, 259, 189, 136, 135 and 121. (Found: C, 77·25, H, 10·5. $C_{24}H_{38}O_3$ Requires: C, 77·1; H, 10·2%.) This gave a 2,4-dinitrophenylhydrazone, m.p. 285°, and an isomer, m.p. 273°. Neither the acetate (Va) nor its corresponding alcohol (Vb) gave a positive Zimmerman test. Treatment of the alcohol with strong base did not cause epimerization, as might be expected for a 17-ketone.[15] Addition of $Eu(fod)_3$[16] to a solution of the alcohol in $CDCl_3$ caused only two of the methyl groups to move downfield rapidly, by NMR.

Mild acid hydrolysis. A portion of the EtOH extract (420 g) was treated with N HCl, before chromatography through alumina to afford two new compounds, obtained as a mixture. Acetylation, followed by preparative TLC (SiO_2; (1:9), EtOAc–light petrol. 3 elutions) and several recrystallizations (EtOH and MeOH), afforded the two homologous sapogenins, as monoacetates (VII) and (VIII). The major component had m.p. 198–200°, $[\alpha]_D^{25}$ −75·8° (*c* 1·00, $CHCl_3$), no λ_{max}, ν_{max}^{Nujol} 3500, 1735 (strong), 1260, 1180, 1040, 985 cm^{-1}, 5·55 (1H, *m*), 6·50 (1H, *m*), 8·00 (MeCO), 8·77 (Me, *s*), 8·83 (Me, *s*), 9·00 (2× Me, *s*), 9·07 (Me, *s*), 9·10 (Me, *s*), 9·10 (Me, *d*, *J* 6 Hz), 9·20 (2× Me, *s*), *m/e* 530 (M^+) (very weak), 512, 417 (strong), 314 (strong), 299, 271, 259, 257, 189. (Found: 512·3842. $C_{33}H_{52}O_4$ Requires: 512·3865.) (Found: C, 74·6 H, 10.4. $C_{33}H_{54}O_5$ Requires: C, 74·7; H, 10·2%.) For details of MS fragmentations see Scheme 1. Treatment of either the acetate or the free alcohol with 1 N NaOH at reflux for 20 min afforded the ketone (V), identical by TLC, m.p., m.m.p. and spectral comparison. Treatment of either the acetate or the free alcohol with 2·5 N HCl at reflux for 2 hr gave the cyclopentenone (III), again identical by comparison to the authentic sample isolated previously. The minor isomer, isolated from the preparative TLC separation of the initial hydrolysate, was the acetate (VIII), m.p. 168–175°, $[\alpha]_D^{25}$ −39·6° (*c* 0·50, $CHCl_3$), ν_{max}^{Nujol} 3500, 1735, 1260, 1040, 985 cm^{-1} τ 5·50 (1H, *m*), 6·5 (1H, *m*), 8·00 (MeCO), 8·72 (Me, *s*), 8·77 (Me, *s*), 8·88 (Me, *s*), 8·90 (Me, *s*), 9·01 (Me, *s*), 9·05 (Me, *d*, *J* 6 Hz), 9·1 (2 × Me, *s*); *m/e* 516 (M^+) (very weak), 498, 417, 314, 299, 271, 259, 257, 189. (Found: C, 73·9; H, 10·2. $C_{32}H_{52}O_5$ Requires: C, 74·2; H, 10·1%.)

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