Phytochemistry, 1978, Vol. 17, pp. 491–494 Pergamon Press Printed in England.

TRITERPENOID AND PHENOLIC CONSTITUENTS OF *COLUBRINA GRANULOSA*

JAMES N. ROITMAN and LEONARD JURD

Western Regional Research Center, Agricultural Research Service U.S. Department of Agriculture, Berkeley, CA 94710, U.S.A.

(*Received* 15 *July* 1977)

Key Word Index—*Colubrina granulosa*; Rhamnaceae; heartwood; triterpene acids; polyphenol; maesopsin; ceanothic acid; granulosic acid; colubrinic acid.

Abstract—Ether extracts of *Colubrina granulosa* heartwood yield the polyphenolic coumaranone maesopsin and 3 pentacyclic triterpene acids of the lup-20(29)-en-28-oic acid group, alphitolic, betulinic and ceanothic acids. Two new compounds, granulosic and colubrinic acids, which belong to a very small class of A ring contracted triterpenes were also isolated. The co-occurrence of maesopsin, alphitolic, betulinic and ceanothic acids suggests that *C. granulosa* (from Panama) may be closely related or identical to the Australian species, *Alphitonia whitei*, in which these relatively rare compounds also occur.

RESULTS AND DISCUSSION

As part of a continuing study of chemical constituents from marine borer resistant woods the heartwood of the Panamanian tree *Colubrina granulosa* Perk. (Rhamnaceae) was examined. The water soluble portion of the ether extract yielded a large amount of a phenolic compound identified as maesopsin, [2,4,6-trihydroxy-2-(4-hydroxybenzyl)-coumaran-3-one], previously isolated from *Maesopsis eminii* [1] and *Alphitonia whitei* [2]. The mp and IR spectra of the *Colubrina* polyphenol and its tetraMe ether closely agree with those reported [1,2] for maesopsin and its tetraMe ether. This identification was confirmed* by the PMR spectrum (dioxane) of the *Colubrina* phenol which shows in addition to 4 OH groups and a phloroglucinol ring system two A_2B_2 doublets at δ 6.56 and δ 7.04 ($J = 8$ Hz), and a 2H singlet at δ 3.03 corresponding to the aromatic and benzylic protons of the 4-hydroxybenzyl group respectively.

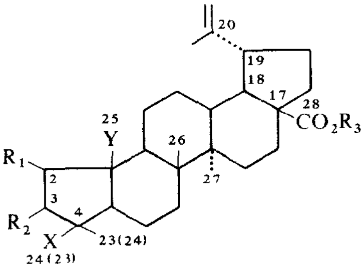
Five triterpene acids were separated from the water-insoluble fraction of the heartwood ether extract by successive extraction with $NaHCO_3$, Na_2CO_3 and NaOH. Acidification of the Na_2CO_3 solution gave major amounts of ceanothic acid **1**, identical in all respects with an authentic specimen.

The precipitate which formed during Na_2CO_3 extraction was esterified with ethereal diazomethane and the crude product recrystallized yielding pure Me betulinate identified by its mp, PMR, MS and IR spectra with reported values [3, 4]. Chromatography of the mother liquors of Me betulinate on silica gel gave in addition to more Me betulinate, fractions much more and much less polar. Crystallization of the more polar fraction gave a small amount of Me alphitolate identified by MS, PMR and IR spectra which agree with reported values [2, 3, 5] although we observed a mp 243–245°, somewhat higher than that reported (233–235° [2], 234–237° [5]). The less polar fraction gave on recrystallization a solid mp 53–55°, shown by GC–MS to be a homologous series of Me esters of C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, C_{28} and C_{30} acids with smaller amounts of the corresponding odd carbon acid Me esters. The material in the mother liquor from this crystalline mixture was separated into 3 components by preparative-GLC, and identified as the Me esters of palmitic, stearic and a mono-unsaturated C_{18} linear acid, probably oleic.

The $NaHCO_3$ extract gave upon acidification granulosic acid **3** a new triterpene acid, mixed with ceanothic acid. The mixture was converted into the corresponding Me esters and separated by chromatography on silica gel. The MS of Me granulosate **4** establishes its molecular formula as $C_{32}H_{50}O_6$ and, consequently, that of the acid as $C_{30}H_{46}O_6$. The PMR spectrum ($CDCl_3$) is similar to that of Me ceanothate **2** except that diMe granulosate shows only 4 tertiary Me groups (δ 1.32, δ 1.18, δ 0.91 × 2) whereas diMe ceanothate has 5 (δ 1.12, δ 1.07, δ 0.92 × 3), and the C_2 proton of diMe granulosate occurs as an unresolved doublet at α 2.67 ($W_{\frac{1}{2}} =$ 1.5 Hz) whereas that of **2** occurs at δ 2.60 ($W_{\frac{1}{2}} \sim 2$ Hz). The low coupling constant $J_{23} < 1.2$ Hz indicates that the stereochemical disposition of the substituents on C_2 and C_3 are α and β respectively as in ceanothic acid [6]. The presence of OH groups, indicated from upfield shifts of two signals (δ 3.47 → δ 3.33 and δ 2.58 → δ2.41) caused by warming to 50° and from absorptions in the IR spectrum at 3515 cm^{-1} (sharp) and 3350 cm^{-1}, was confirmed by the formation of a diacetate **5** by treatment of **4** with acetic anhydride and pyridine. Coupled with the fact that the molecular formula of **4** indicates 8 degrees of unsaturation, 5 of which are accounted for by rings, one by a double bond and the additional two by carbonyl groups of the two carboxymethyl groups, this indicates that granulosic acid is derived from ceanothic acid by replacement of one of the tertiary Me groups by a hydroxymethyl group. The MS shows several fragments

---

* MS and PMR spectra of maesopsin and its tetramethyl ether do not appear to have been previously reported.



1 $R_1 = \alpha\text{-}CO_2H$; $R_2 = \beta\text{-}OH$; $R_3 = H$; X = Y = Me
2 $R_1 = \alpha\text{-}CO_2Me$; $R_2 = \beta\text{-}OH$; R_3 = X = Y = Me
3a $R_1 = \alpha\text{-}CO_2H$; $R_2 = \beta\text{-}OH$; $R_3 = H$; X = $\beta\text{-}CH_2OH$; Y = Me
3b $R_1 = \alpha\text{-}CO_2H$; $R_2 = \beta\text{-}OH$; $R_3 = H$; X = $\alpha\text{-}CH_2OH$. Y = Me
3c $R_1 = \alpha\text{-}CO_2H$;$R_2 = \beta\text{-}OH$; $R_3 = H$; X = Me; Y = CH_2OH
4a $R_1 = \alpha\text{-}CO_2Me$; $R_2 = \beta\text{-}OH$, R_3 = Y = Me; X = $\alpha\text{-}CH_2OH$
4b $R_1 = \alpha\text{-}CO_2Me$, $R_2 = \beta$ OH; R_3 = Y = Me; X = $\beta\text{-}CH_2OH$
4c $R_1 = \alpha\text{-}CO_2Me$; $R_2 = \beta\text{-}OH$; R_3 = X = Me; Y = CH_2OH
5a $R_1 = \alpha\text{-}CO_2Me$, $R_2 = \beta\text{-}OAc$; R_3 = Y = Me; X = $\alpha\text{-}CH_2OAc$
5b $R_1 = \alpha\text{-}CO_2Me$; $R_2 = \beta\text{-}OAc$; R_3 = Y = Me; X = $\beta\text{-}CH_2OAc$
5c $R_1 = \alpha\text{-}CO_2Me$; $R_2 = \beta\text{-}OAc$, R_3 = X = Me; Y = CH_2OAc
6a $R_1 = \beta\text{-}CHO$; $R_2 = \beta\text{-}OH$; $R_3 = H$; X = Y = Me
6b $R_1 = \beta\text{-}CHO$; $R_2 = \alpha\text{-}OH$; $R_3 = H$; X = Y = Me
6c $R_1 = \alpha\text{-}CHO$, $R_2 = \alpha\text{-}OH$; $R_3 = H$; X = Y = Me
7a $R_1 = \beta\text{-}CHO$; $R_2 = \beta\text{-}OH$; R_3 = X = Y = Me
7b $R_1 = \beta\text{-}CHO$; $R_2 = \alpha\text{-}OH$; R_3 = X = Y = Me
7c $R_1 = \alpha\text{-}CHO$; $R_2 = \alpha\text{-}OH$; R_3 = X = Y = Me
8a $R_1 = \beta\text{-}CHO$; $R_2 = \beta\text{-}OAc$; R_3 = X = Y = Me
8b $R_1 = \beta\text{-}CHO$; $R_2 = \alpha\text{-}OAc$, R_3 = X = Y = Me
8c $R_1 = \alpha\text{-}CHO$; $R_2 = \alpha\text{-}OAc$; R_3 = X = Y = Me

which can be attributed to segments containing rings D and E, notably *m/e* 262, 233, 203, 189 and 175. The same fragments occur in the spectra of diMe ceanothate, Me alphitolate, Me betulinate and another new triterpene acid Me ester described below, suggesting that the oxygenated Me group in **4** is not C-27.

In addition the chemical shifts of the two methylene protons (located by double resonance experiments) occur at δ4.22 and δ3.28 a chemical shift difference which can only be explained by restricted rotation of the CH_2OH group probably caused by hydrogen bonding of the OH to a nearby carbomethoxy group. This notion is further supported by the much lower chemical shift difference for the CH_2OAc methylene protons in the diacetate **5** (δ4.28 and δ4.03). The C-26 Me group is too distant from the hydrogen bonding sites to be considered, leaving the 3 Me groups attached to the 5 membered A ring namely C-23 C-24 and C-25. In the absence of definitive proof which may be provided by chemical modifications of **4** but which have not yet been attempted because of scarcity of the material, we propose structures **3a**, **3b** or **3c** for granulosic acid until more information is available.

A second new triterpene acid, colubrinic acid, was isolated as its Me ester by esterification of the insoluble sodium salt which formed during NaOH extraction. On the basis of the molecular formula ($C_{31}H_{48}O_4$) of Me colubrinate, colubrinic acid **6** is a monocarboxylic acid, $C_{30}H_{46}O_4$. The PMR spectrum of Me colubrinate is similar to that of Me ceanothate except that it shows only one carbomethoxy group and in addition a proton as a doublet (J = 3.5 Hz) at low field (δ 9.93) indicative of an aldehyde attached to secondary carbon. This was confirmed by the IR absorption at 1715 cm^{-1}. A proton which appears at δ4.29 as a doublet (J = 8.5 Hz) suggests a carbinol, confirmed by formation of a monoacetate **8** in which the doublet is shifted to δ5.4. A double resonance experiment established that both the aldehyde proton and the carbinol proton are coupled to a single proton which appears at δ2.14 as a pair of doublets partially obscured by overlapping resonances. This suggest that the 3 protons are attached to contiguous carbon atoms as in **7**; the large coupling constant between the protons attached to C-2 and C-3, J = 8.5 Hz, on the other hand indicates a stereochemical arrangement other than that of ceanothic and granulosic acid.

The lack of correlation between vicinal coupling constants and configuration of 5 membered rings [7] makes it impossible to specify the stereochemical arrangement of the groups attached to C-2 and C-3. Attempts to convert the aldehyde group of **7** to a carbomethoxy group have not been successful. The PMR spectra of diMe ceanothate **2** and of its C-2 and C-3 epimers have characteristics allowing positive identification of any one of them [6]. Of the 4 epimers only ceanothic acid and its derivatives have a low C-2–C-3 coupling constant and for this reason we have ruled out the 2α, 3β isomer of **6**, **7** and **8**.

The occurrence in *C. granulosa*, a tree of Panamanian origin, of maesopsin and ceanothic betulic and alphitolic acids, all of which have also been found [2] in the Australian timber, *A. whitei* (Rhamnaceae) indicates that the botanical classification of these two species needs to be re-examined, since these compounds are relatively rare natural products. In this connection it is noteworthy that *C. excelsa* has been shown to be identical to *A. excelsa* [9] and *C. buxifola* is identical with *Ceanothus buxifolus* [8].

EXPERIMENTAL

All mps are uncorr. MS were obtained at 70 eV by electron impact. PMR spectra were obtained at 100 MHz in $CDCl_3$ and are reported in ppm δ downfield from internal TMS. Column chromatography was performed with Si gel and TLC on precoated Si gel plates visualized with phosphomolybdic acid spray followed by heating. IR spectra were obtained in KBr pellets or as Nujol mulls.

Extraction of Colubrina granulosa. Hammermilled heartwood chips (4 kg) were extracted with boiling Et_2O for 7 days. The crude extract was concd to small vol. boiled with 2l. petrol and dried after the petrol solubles had been removed. The yellow powder which remianed (95 g) was warmed with 3 l. of H_2O for 1 hr then filtered yielding 47 g of H_2O insoluble light yellow powder (A) containing triterpenoids and a dark aq. soln.

Isolation of maesopsin. The aq. soln described above was evapd to a hard brownish crystalline mass which was digested with warm EtOAc. The insoluble solid was recrystallized from H_2O to give maesopsin as large colorless prisms, mp 219–220°, (32.2 g). (Found . C, 62.8; H, 4.26. Calc. for $C_{15}H_{12}O_6$. C, 62.5; H, 4.20); MS obs. *m/e* 288.0632. Calc. for $C_{15}H_{12}O_6$. 288.0638; IR λ_{max}^{Nujol} 1630, 1685, 3200 cm^{-1}. MS *m/e* (rel int.) 288 (2), 270 (16), 242 (6), 182 (5), 181 (9), 153 (17), 108 (24), 107 (100). PMR (d_8 dioxane) 3.03 (2H, *s*), 5.7 (1H, *d*, $J = 2$ Hz), 5.81 (1H, *d*, $J = 2$ Hz), 6 (1H, *s*, OH), 6.56 (2H, *d*, $J = 8$ Hz), 7.04 (2H, *d*, $J = 8$ Hz), 7.33 (1H, *s*, OH), 8.47 (1H, *s*, OH), 8.68 (1H, *s*, OH). With Me_2SO_4, K_2CO_3 and Me_2CO the product formed a tetraMe ether which crystallized from MeOH as colorless prisms, mp 130–131° (lit. [2] mp for maesopsin tetraMe ether 130–132° and 118° [dimorphic]). Found. C, 66.3; H, 6.02 Calc. for $C_{19}H_{20}O_6$. C, 66.3; H, 5.85. PMR. 3.11 (1H,*s*), 3.14 (1H,*s*), 3.28 (3H,*s*), 3 76 (3H,*s*), 3.85 (3H,*s*), 3.87 (3H, *s*), 5.91 (1H, *d*, $J = 2$ Hz), 6.08 (1H, *d*, $J = 2$ Hz), 6.73 (2H, *d*, $J = 8$ Hz), 7.17 (2H, *d*, $J = 8$ Hz).

Base extraction of the acidic constituents from A. The H_2O and petrol insoluble material A was dissolved in 3 l. of Et_2O and extracted with (1) 8 % $NaHCO_3$ (3 × 650 ml) which gave on acidification B (0.5 g), (2) 8 % Na_2CO_3 (3 × 650 ml) which gave after acidification C (28 g), (3) 10 % NaOH (500 ml) which gave after acidification D (0.2 g). The residual Et_2O soln yielded brown semisolid E (9 g). During Na_2CO_3 extraction a ppt. formed which was collected by filtration over diatomaceous earth, acidified with dil. HCl and extracted into Et_2O giving F (2.7 g). A second ppt. was obtained during NaOH extraction and similar handling yielded G (3.6 g).

2α-Carboxy-3β-hydroxy-nor-A(1)-lup-20(29)-en-28-oic acid. (*Ceanothic acid 1*). The crude material C from the Na_2CO_3 extract was twice recrystallized from Me_2CO–MeOH after treatment with decolorizing carbon giving colorless fine needles of pure ceanothic acid mp 333–335°, mmp 332–335° (sealed capillary). PMR (d_5-Py): 1.06 (3H, *s*, Me), 1.14 (3H, *s*, Me), 1.25 (3H, *s*, Me), 1.37 (3H, *s*, Me), 1.4 (3H, *s*, Me), 1.65 (3H, *s*, vinyl Me), 3.16 (1H, *br s*, C-2-H), 3.46 (1H, *br m*, C-19-H), 4.64 (1H, *m*, vinyl), 4 77 (1H, *br s*, C-3-H), 4.84 (1H, *m*, vinyl). MS *m/e* (rel. int.): 486 (M^+, 20), 468 (M^+-H_2O, 13), 440 (M^+-H_2O—CO, 25), 248 (37), 237 (22), 234 (16), 219 (29), 205 (23), 203 (26), 189 (48), 175 (52), 147 (34), 133 (43), 121 (80). IR ν_{max}^{Nujol} : 3470 (OH), 1687 (C=O), 885 ($C{=}CH_2$) cm^{-1}.

Me-2α-carboxymethyl-3β-hydroxy-nor-A(1)-lup-20(29)en-28-oate (*diMe ceanothate 2*). A MeOH soln of ceanothic acid **1** was treated with excess CH_2N_2–Et_2O at 0° for 1 hr, the solvents removed and the residue recrystallized from MeOH which gave hard colorless prisms, mp 219–221° (lit. [10] mp 223°). PMR: 0.94 (9H, *s*, 3 × Me), 1.08 (3H, *s*, Me), 1.14 (3H, *s*, Me), 1.68 (3H, *s*, vinyl Me), 1.95 (1H, *d*, $J = 4$ Hz, OH), 2.61 (1H, *br s*, C-2-H), 2.96 (1H, *br m*, C-19-H), 3.68 (3H, *s*, OMe), 3.7 (3H, *s*, OMe), 4.17 (1H, *d*, $J = 4$ Hz C-3-H), 4.59 (1H, *m*, vinyl), 4.72 (1H, *m*, vinyl). MS *m/e* (rel. int.). 514 (M^+, 16); 262 (18); 251 (23); 233 (20); 203 (32); 189 (49); 175 (55); 159 (25); 147 (33); 133 (57); 121 (77). IR ν_{max}^{KBr} . 3530 (OH), 1730, 1713 (C=O), 890 ($C{=}CH_2$) cm^{-1}. $[\alpha]_D^{25} = +47°$ (*c* 0.3 $CHCl_3$); lit. [11] $[\alpha]_D^{18} = 45°$ (*c* 1.4 $CHCl_3$).

Me-3β-hydroxylup-20(29)-en-28-oate(Me betulinate). The material F (3.8 g) which pptd during Na_2CO_3 extraction was dissolved in Et_2O–THF and treated with excess CH_2N_2–Et_2O at 0° for 15 min then quenched with HOAc. The residue remaining after removal of solvents was recrystallized after decolorizing charcoal treatment from Me_2CO–C_6H_6 giving 0.8 g of fine colorless needles of Me betulinate, mp 229–231°; lit [4] mp 222–224°. $[\alpha]_D^{25} = 0°$, $[\alpha]_{313}^{25} = +20°$ (*c* 0.4 $CHCl_3$); lit. [4] $[\alpha]_D = +5$ (*c* 0.7 $CHCl_3$). PMR: 0.76 (3H, *s*, Me), 0.82 (3H, *s*, Me), 0.92 (3H, *s*, Me), 0.97 (6H, *s*, 2 × Me), 1.69 (3H, *s*, vinyl Me), 3.05 (1H, *br m*, C-19-H), 3.68 (3H, *s*, OMe), 4.6 (1H, *m*, vinyl), 4.74 (1H, *m*, vinyl). Lit. [3] PMR for Me groups. δ 0.76, 0.83, 0.92, 0.96 × 2, 1.7. MS *m/e* (rel. int.). 470 (M^+, 17), 452 (M^+-H_2O, 6) 411 (M^+-CO_2Me, 9), 262 (27), 233 (10), 220 (20), 207 (39), 203 (31), 189 (92), 175 (44), 147 (26), 135 (47), 119 (58). IR ν_{max}^{KBr} . 3325 (OH), 1730 (C=O), 887 (C=CH) cm^{-1}.

Me n-*alkanoates.* The mother liquor from which Me betulinate had crystallized was chromatographed on Si gel employing 5 % Et_2O in C_6H_6 as eluant. The first materials eluted were homogeneous by TLC (Et_2O–petrol, 1.24) and were recrystallized from MeOH and then from Me_2CO–MeOH (1:1) giving colorless crystalline plates (31 mg) mp 53–55° IR ν_{max}^{Nujol} : 1745 cm^{-1} (C=O). PMR 0.88 (3H, *t*, $J = 6$ Hz, terminal Me), 1.26 [*ca* 36H, *br s*, $(CH_2)_n$], 1.5 (2H, *br m*, CH_2-CH_2CO), 2.3 (2H, *t*, CH_2CO), 3.68 (3H, *s*, OMe). GC–MS (1 m × 2 mm OV-1 on Chromosorb W) gave 12 distinct peaks the spectra of which were characteristic of Me *n*-alkanoates commencing with Me octadecanoate ($C_{19}H_{38}O_2$, $M^+ = 298$) and terminating with Me triacontanoate ($C_{31}H_{62}O_2$, $M^+ = 466$). The spectra of the intermediate peaks corresponded to the Me esters of C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28} and C_{29} *n*-alkanoic acids. When the mother liquor of the above mixture of solid esters was subjected to GLC (1 m × 2 mm OV-1 on Chromosorb W, 195°), 3 compounds were isolated in a pure state. A (R_t 25 min) and C (R_t 55 min) possessed identical PMR spectra to the mixture of solid Me esters isolated previously thus indicating that they were Me *n*-alkanoates. MS *m/e* M^+. A 270; C 298. Therefore A is Me hexadecanoate and C is Me octadecanoate. Compound B (R_t 45 min) shows M^+ 296 and a PMR spectrum which corresponds to a Me ester of C_{18} *n*-alkenoic acid. 0.89 (3H, *t*, $J =$ 6 Hz, terminal Me, 1.31 [*ca* 22H, *br s*, $(CH_2)_n$], 1.5 (2H, *m*, $C\underline{H}_2CH_2CO$), 2.04 (4H, *m*, 2 × CH_2—CH=), 2.31 (2H, *t*, $J = 7$ Hz, CH_2CO), 3.66 (3H, *s*, OMe), 5.35 (2H, *m*, vinyl).

Me 2α, 3β-dihydroxylup-20(29)-en-28-oate (*Me alphitolate*). Further elution of the chromatographic column with increasing concns of Et_2O in C_6H_6 resulted in isolation of more Me betulinate (10 % Et_2O), traces of unidentified substances, and finally (50 % Et_2O) of a substance much more polar than Me betulinate (230 mg). This material was rechromatographed on Si gel employing $CHCl_3$ as eluant to give some material which was nearly homogeneous by TLC and which was purified by two recrystallizations from MeOH giving colorless needles of Me alphitolate (55 mg) mp 243.5–245°. *IR* ν_{max}^{KBr}. 3300 (OH), 1725 (C=O), 890 ($C{=}CH_2$) cm^{-1} PMR: 0.8 (3H, *s*, Me), 0.89 (3H, *s*, Me), 0.92 (3H, *s*, Me), 0 96 (3H, *s*, Me), 1.01 (3H, *s*, Me), 1.68 (3H, *s*, vinyl Me), 2.97 (1H, *dd*, $J_{2,3} = 10$ Hz, $J_{3,OH} = 4$ Hz, C-3-H), 3 (1H, *br m*, C-19-H), 3.52 (1H, *s*, OH), 3.67 (3H, *s*, OMe), 3.65 (1H, *br m*, C-2-H), 4.61 (1H, *m*, vinyl), 4.74 (1H, *m*, vinyl). PMR (d_5-Py): 0.95 (3H, *s*, Me), 1 (6H, *s*, 2 × Me), 1.08 (3H, *s*, Me), 1.26 (3H, *s*, Me), 1.75 (3H, *s*, vinyl Me), 3.3 (1H, *br m*, C-19-H), 3.37 (1H, *d*, $J = 9$ Hz, C-3-H), 3.72 (3H, *s*, OMe), 4.08 (1H, *br m*, C-2-H), 4.76 (1H, *m*, vinyl), 4.9 (1H, *m*, vinyl). Lit [3] PMR for Me groups ($CDCl_3$). δ 0.8, 0.9, 0.92, 0.97, 1.01, 1.7. MS *m/e* (rel. int.). 486 (M^+, 18), 468 (M^+-H_2O, 8), 426 (M^+-CO_2Me, 13), 409 (M^+-CO_2Me-H_2O, 11), 262 (44), 249 (39), 236 (16), 233 (16), 223 (35), 215 (10), 205 (40), 203 (41), 189 (99), 175 (42), 147 (24), 133 (32), 119 (41)

Me 2-formyl-3-hydroxy-nor-A(1)-lup-20(29)-en-28-oate (*Me colubrinate*) **7**. One portion (3 g) of the ppt. (G) which formed during NaOH extraction was dissolved in Et_2O–THF and treated with excess CH_2N_2–Et_2O at 0° for 2 hr and allowed to stand 18 hr. A TLC of the crude product when sprayed with 2,4-DNPH no longer showed a positive reacting spot suggesting that the aldehyde had been destroyed by reaction with CH_2N_2. Accordingly the remainder of G (0.6 g) was treated at 0° with CH_2N_2 for only 15 min, quenched with HOAc and the residue remaining after removal of solvents was chromatographed on Si gel employing C_6H_6–Et_2O as eluant. After fractions containing fatty acid esters and Me betulinate had been collected a more polar material giving a positive 2,4-DNPH reaction was eluted with 10% Et_2O Recrystallization of this material from Et_2O–petrol gave hard cubic crystals of Me colubrinate (107 mg) mp 173–180° *d* (gas evolution). $[\alpha]_D^{25} = -39°$ (*c* 0.2 $CHCl_3$). IR ν_{max}^{KBr} . 3540 (OH). 1725 (C=O), 1715 (C=O), 890 ($C{=}CH_2$) cm^{-1}. M^+ *m/e* obs.. 484.3552; $C_{31}H_{48}O_4$ requires 484.3552. PMR: 0.9 (3H, *s*, Me), 0.94 (3H, *s*, Me), 0.98 and 0 99 (9H, 2*s*, 3 × Me), 1 68 (3H, *s*, vinyl Me), 2.14 (1H, *dd*, *J* = 8.5 Hz, *J* = 3.5 Hz, C-2-H), 2.96 (1H, *br m*, C-19-H), 3.68 (3H, *s*, OMe), 4.29 (1H, *d*, *J* = 8.5, C-3-H), 4.61 (1H, *m*, vinyl), 4.73 (1H, *m*, vinyl), 9.93 (1H, *d*, *J* = 3.5, CH=O). MS *m/e* (rel. int): 484 (M^+, 36), 466 (M^+-H_2O, 23), 425 (M^+-CO_2Me, 16), 273 (11), 262 (34), 249 (18), 233 (10), 215 (13), 203 (51), 189 (100), 175 (66), 161 (33), 147 (36), 145 (36), 133 (59), 121 (83), 119 (78)

Me 2-formyl-3-acetoxyl-nor-A(1)-lup-20(29)-en-28-oate (*Me colubrinate acetate*) **8** 10 mg of Me colubrinate **7** was warmed with 0 5 ml of Ac_2O and one drop of Py for 20 min, diluted with H_2O, allowed to stand for 3 hr and extracted with Et_2O Removal of Et_2O gave a residue which crystallized from MeOH (0.5 ml) as colorless fine needles (4 mg) mp 172–180° M^+ *m/e* obs. 526.3682; $C_{33}H_{50}O_4$ requires 526.3658. PMR. 0 86 (3H, *s*, Me), 0.91 (3H, *s*, Me), 1 (3H, *s*, Me), 1.06 (3H, *s*, Me), 1 1 (3H, *s*, Me), 1 69 (3H, *s*, vinyl Me), 2.03 (3H, *s*, MeCO), 2.35 (1H, *dd*, *J* = 8.5 Hz, *J* = 3.5 Hz, C-2-H), 2 96 (1H, *br m*, C-19-H), 3.68 (3H, *s*, OMe), 4.62 (1H, *m*, vinyl), 4.74 (1H, *m*, vinyl), 5.4 (1H, *d*, *J* = 8.5 Hz, C-3-H), 9.82 (1H, *d*, *J* = 3.5 Hz, CH=O). MS *m/e* (rel int.): 526 (M^+, 4), 508 (M^+-H_2O, 4), 466 (M^+-$MeCO_2H$, 9), 433 (6), 407 (4), 273 (5), 262 (15), 249 (7), 229 (8), 215 (14), 203 (29), 189 (59), 175 (44), 161 (17), 149 (20), 133 (29), 121 (42), 119 (36).

DiMe granulosate **3**. The acidified $NaHCO_3$ extract B (500 mg) was dissolved in Et_2O–THF, treated with excess CH_2N_2–Et_2O at 0° for 15 min, quenched with HOAc and after removal of solvents chromatographed on Si gel. The major constituent which followed some Me ceanothate was eluted with 20% Et_2O in C_6H_6. Crystallization of the amorphous solid (350 mg) from MeOH gave colorless needles of diMe granulosate (270 mg) mp 221–222°. $[\alpha]_D^{25} = +60$ (*c* 0.2 $CHCl_3$). M^+ obs. *m/e* 530.3617; $C_{32}H_{50}O_6$ requires. 530.3607. IR ν_{max}^{KBr} . 3515 (OH), 3350 (OH), 1730 (C=O), 895 ($C{=}CH_2$) cm^{-1}. PMR. 0.9 (6H, *s*, 2 × Me), 1.18 (3H, *s*, Me), 1.32 (3H, *s*, Me), 1.66 (3H, *s*, vinyl Me), 2.58 (1H, *dd*, J_{AM} = 7 Hz, J_{AX} = 3 Hz, $CH_MH_XO\underline{H}_A$), 2.67 (1H, unresolved *d* $W_{\frac{1}{2}}$ = 1.5 Hz, C-2-H), 2.96 (1H *br m*, C-19-H), 3.28 (1H, *dd*, J_{AM} = 7 Hz, J_{MX} = 11 Hz, $C\underline{H}_MH_XOH_A$), 3.47 (1H, *d*, *J* = 2Hz, C-3-OH), 3.67 (3H, *s*, OMe), 4.22 (1H, *dd*, J_{AX} = 3 Hz, J_{MX} = 11 Hz, $CH_M\underline{H}_XOH_A$), 4.28 (1H, *d*, *J* = 2 Hz, C-3-H), 4 61 (1H, *m*, vinyl), 4.74 (1H, *m*, vinyl). MS *m/e* (rel. int.). 530 (M^+, 39), 512 (M^+–H_2O, 11), 498 (42), 480 (18), 471 (M^+-CO_2Me, 16), 470 (25), 450 (35), 439 (16), 421 (13), 416 (12), 316 (13), 281 (23), 273 (75), 267 (39), 262 (77), 249 (29), 248 (25), 247 (25), 233 (39), 220 (90), 203 (84), 189 (100), 187 (73), 177 (44), 175 (86), 173 (59), 161 (39), 159 (35), 147 (45), 145 (37), 135 (40), 134 (40), 133 (61), 131 (36), 121 (67), 119 (96).

DiMe granulosate diacetate **5** A sample of di Me granulosate **4** (50 mg) was warmed with 1 ml of Ac_2O and 2 drops of Py for 20 min, diluted with H_2O and collected after standing 18 hr. Crystallization from MeOH–H_2O gave diMe granulosate diacetate **5** as colorless hard crystals (38 mg) mp 148–149 . PMR. 0.92 (6H, *s*, 2 × Me), 1 08 (3H, *s*, Me), 1.32 (3H, *s*, Me), 1.68 (3H, *s*, vinyl Me), 2.01 (6H, *s*, 2 × MeCO), 2.62 (1H, unresolved *d* $W_{\frac{1}{2}}$ = 1.5 Hz, C-2-H), 2 96 (1H, *br m*, C-19-H), 3.68 (3H, *s*, OMe), 3.7 (3H, *s*, OMe), 4.02 (1H *d*, *J* = 11 Hz, CH_AH_BOAc), 4.28 (1H, *d*, *J* = 11 Hz, CH_AH_BOAc), 4.62 (1H, *m*, vinyl), 4.73 (1H, *m*, vinyl), 5.26 (1H, *br s*, C-3-H). MS *m/e* (rel. int.) 614 (M^+, 48), 582(35), 554 (M^+-$MeCO_2H$, 20), 522 (12), 354 (11), 305 (10), 273 (15), 262 (32), 248 (15), 233 (15), 219 (10), 203 (24), 189 (40), 187 (29), 177 (15), 175 (26), 173 (25), 161 (13), 159 (16), 147 (16), 145 (17), 135 (13), 133 (25), 131 (15), 121 (24), 119 (39)

Acknowledgements—We are indebted to Dr. R. Eade for a sample of authentic ceanothic acid and to Miss G Secor, Dr. W. Haddon and Mrs. M Wiley for elemental analyses and measurement of MS and PMR

REFERENCES

1. Janes, N. F., King, F. E. and Morgan, J. W. W. (1961) *Chem. Ind.* (*Lond.*) 346.
2. Guise, G. B., Ritchie, E and Taylor, W. C. (1962) *Australian J. Chem.* **15**, 314.
3. Cheung, H. T. and Williamson, D G. (1969) *Tetrahedron* **25**, 119.
4. Alpin, R. T., Halsall, T. G. and Norin, T. (1963) *J Chem. Soc.* 3269.
5. Cheung, H. T. and Feng, M. C. (1968) *J Chem. Soc.* 1047.
6. Eade, R. A., Grant, P. K., McGrath, M. J A., Simes, J. J. H. and Wootton, M. (1971) *Australian J Chem.* **24**, 621
7. Jackman, L. M. and Sternhell, S. (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd edn, p. 287. Pergamon Press, Oxford
8. *Index Kewensis* Vol. I, p. 586.
9. *Benth Fl. Austral.* **1**, 414 (1863); *Index Kewensis*-Supplement XII (1951–1955).
10. Julian, P. L., Pikl, J. and Dawson, R. (1938) *J. Am. Chem. Soc.* **60**, 77.
11. Birch, A. J., Ritchie, E. and Speake, R N. (1960) *J Chem Soc* 3593