# FUNGAL METABOLITES. IX[1]. TRITERPENES FROM *NAEMATOLOMA SUBLATERITIUM*

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ABSTRACT.—Fasciculol B (1), fasiculol C (**2**) and their depsipeptides (3–4) have been identified in fruit bodies of *Naematoloma sublateritium* by structure determination and hplc. Chemotaxonomic relationship with other Naematoloma species is reported.

*Naematoloma sublateritium* (Schaefer ex Fr.) Karst. is a toxic mushroom of the genus Jantinosporei (Agaricaceae, Basidiomycetes) which grows in tufts on the stumps of old trees (1).

Previous chemical investigations on fruit bodies led to the isolation of ergosterol (2) and the pigments named fasciculines and hypholomines (3). In addition, studies on the aminoacids (4), hydrocarbons (5), lipids (6), and inorganic elements (7) in the fruit bodies have also been reported.

Pursuing our research on terpene metabolites from Basidiomycetes, we examined the acetone extract of fresh fruit bodies of *N. sublateritium*; we isolated four triterpene compounds (**1–4**) along with ergosterol, ergosterol peroxide and cerevisterol.

On the basis of spectroscopic data and chemical evidence, we assigned to **1–4** the following structures: (**1**) 2$\alpha$,3$\beta$,12$\alpha$,24R,25-pentahydroxylanosta-8-ene, (**2**) 2$\alpha$,3$\beta$,12$\alpha$,21,24R,25-hexahydroxylanosta-8-ene, (**3**) 3$\beta$,12$\alpha$,24R,25-tetrahydroxylanosta-8-ene 2$\alpha$(N-glycyl methyl ester)-3'-hydroxy-3'-methylglutarate, and (**4**) 3$\beta$,12$\alpha$,21,24R,25-pentahydroxylanosta-8-ene 2$\alpha$(N-glycyl methyl ester)-3'-hydroxy-3'-methylglutarate.

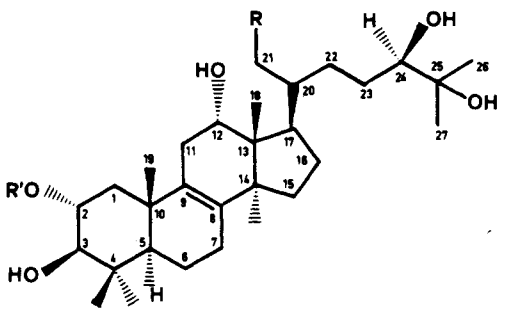
Chemical evidences for **1** and **2** rested on: the formation of a variety of acetyl-derivatives, depending on reaction conditions; the formation of mono- and diacetonides; the cleavage of the chain and ring A by periodic oxidation of **2** followed, in basic conditions, by condensation of the moieties of the seco-ring A to an $\alpha,\beta$-unsaturated aldehyde (**5**); the epoxidation of the double bond followed by acidic opening of the oxirane ring affording the characteristic 7,8–9,11-diene system (uv); and for **3** and **4** the hydrolysis, respectively, to **1** and **2** and to glycine and 3-hydroxy-3-methylglutaric acid.

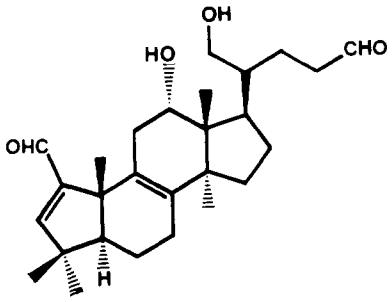
These compounds looked identical to fasciculol B and fasciculol C recently isolated from *Naematoloma fasciculare* (8, 9) and their depsipeptides, which are all plant growth inhibitors.

In order to confirm the identity of **1–4** with fasciculols, we extracted the fruit bodies of *N. fasciculare* collected both in Italy and in Poland; we compared the

[1]For part VIII see R. Battaglia, M. De Bernardi, G. Fronza, G. Mellerio, G. Vidari and P. Vita-Finzi, J. Nat. Products, **43**, 319 (1980).

[2]Deceased on 23 March 1980.





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1 R = R' = H

2 R = OH R' = H

3 R = H R' = CO-$CH_2$-C(OH)(-$CH_3$)-$CH_2$-CONH-$CH_2$-COOCH$_3$

4 R = OH R' = CO-$CH_2$-C(OH)(-$CH_3$)-$CH_2$-CONH-$CH_2$-COOCH$_3$

terpene fraction of both mushrooms by tlc and hplc. The isolation of fasciculols from *N. fasciculare* was proof of the structures for **1–4**.

Separation of **1–4** by hplc could be done only on a reversed phase μBondapack CN column by elution with methanol-water (45:55). By chromatographic methods (hplc and tlc) analysis of an other species of *Naematoloma*, *N. capnoides* excluded the presence of fasciculols in this mushroom.

These data on triterpene content allowed us to infer a chemotaxonomic difference between *N. capnoides* and *N. sublateritium* and *N. fasciculare* as was also indicated by the study of the pigments (3) of eight *Naematoloma* species which showed the same content for all the species except *N. capnoides*. Furthermore it is interesting to note that the two species containing the triterpenes are bitter, while *N. capnoides* is good tasting and edible.

## EXPERIMENTAL[3]

PLANT MATERIAL.—*Naematoloma sublateritium* (Schaefer ex Fr.) Karts. and *Naematoloma capnoides* (Fr.) Karts. were collected near Pavia (Italy), and *Naematoloma fasciculare* (Hudson ex Fr.) Karst. was collected in both Italy and Poland.

ISOLATION OF STEROIDS AND TRITERPENES.—*N. sublateritium* fruit bodies (3 kg) were homogenized in a mixer with acetone and twice extracted with the same solvent. The acetone was evaporated *in vacuo*, and the aqueous solution was extracted three times with ethyl acetate. The residue obtained by evaporation of the ethyl acetate (17.0 g) was chromatographed on an alumina column (500 g) by elution with chloroform and increasing percentages of ethyl acetate and then methanol. The first fractions contained esters of fatty acids. Then ergosterol, ergosterol peroxide and cerevisterol with traces of other unidentified sterols were eluted followed by compounds **1–4** (3.86 g, 0.13%). The sterols were identified by comparison with authentic samples.

[3]Equipment: ir, Perkin Elmer 257; uv, Perkin Elmer 200; nmr, Varian T-90 and Varian XL-100; ms, Du Pont 21-492B, melting point, Fisher-Johns hot plate; hplc, Waters ALC/CPC 244 equipped with an uv detector at 210 nm and Jobin Yvon Chromatospac. Tlc was carried out on silicagel (Merck 60 $GF_{254}$) and spots were visualized by spraying with vanillin-sulfuric acid solution and then heating at 120° for ten minutes. Column chromatography was performed on neutral $Al_2O_3$ (act. III, Merck), on silicagel 60 (0.063–0.200 mm, Merck) or on dry column silicagel (Woelm).

In a similar way the fruit bodies of *N. fasciculare* (3.3 kg) were worked up to obtain the same three sterols along with a mixture of **1–4** (6.72 g, 0.20%)[4]. The same procedure was carried out on a small amount of fruit bodies (0.3 kg) of *N. capnoides*.

Fasciculols were isolated in the following order **3**, **4**, **1** and **2** by chromatography on a dry silica gel column by elution with pure chloroform and increasing percentages of methanol. Compounds **1** and **2** were completely separated from a mixture by preparative liquid chromatography.

The triterpene fractions and the pure **1–4** were compared by hplc. A good separation was afforded by use of a reversed phase μBondapack CN column (Waters) by elution with a mixture of methanol-water (45:55) at 1 ml/min: Rt: **2** 18.5, **1** 22.5, **4** 24.0 and **3** 30.0.

2α,3β,12α,24R,25-PENTAHYDROXYLANOSTA-8-ENE (FASCICULOL B) (**1**).—A green spot was produced by spraying the tlc plate with vanillin dissolved in sulfuric acid. The compound gave the following properties: mp 230–232°[lit. 235° (8)], $[\alpha]^{21}D$ +80.5° (MeOH); ir (KBr): ν max 3440(OH), 1650(C=C), 1070, 1060, 1030(C–O) $cm^{-1}$; ms (15 eV) *m/z* (%): 492(27, $M^+$), 477(3, M–15), 474(17, M–18), 459(85, M–15–18), 456(13, M–2x18), 441(100, M–2x18–15), 438(3, M–3x18), 423(21, M–3x18–15), 416(3, M–18–15–43, high resolution indicated $C_{27}H_{44}O_3$), 405(7), 401(7), 329(8, M-side chain-18, h.r. indicated $C_{22}H_{33}O_2$), 278(3), 143(7), 127(8), 106(9); pmr ($CD_3COCD_3$, 100 MHz): δ 0.65(s, 3H), 0.84(s, 3H), 1.03(s, 3H), 1.06(s, 3H), 1.11(s, 3H), 1.13(s, 6H), 1.15(d, 3H, *J* 6.5 Hz), 2.6(m, 1H, 11-H), 2.93(dd, 1H, $J_{vic}$ 9.0 Hz, $J_{H-OH}$ 3.5 Hz, 3αH), 3.22 (d, 1H, *J* 4.0 Hz, 12αOH), 3.23(m, 1H, 24-H), 3.36(m, 1H, 2βH), 3.36(d, 1H, *J* 5.0 Hz, 24-OH), 3.44(d, 1H, *J* 3.5 Hz, 3βOH), 3.53(d, 1H, *J* 3.5 Hz, 2α-OH), 4.03(m, 1H, $J_{vic}$ 7.0 Hz, $J_{H-OH}$ 4.0 Hz, 12β-H); $^{13}$c-nmr data are reported in table 1.

2α,3β,24-TRIACETATE OF **1**.—Acetylation was carried out with $Ac_2O$ and pyridine at rt. The triacetate gave the following properties: mp 189–190° [lit. 191° (8)]; ir (KBr): ν max 3480(OH), 1740(CO), 1650(C=C), 1250, 1035(C–O) $cm^{-1}$; ms (15 eV) *m/z*(%): 618(10, $M^+$), 600(25, M–18), 585(100, M–18–15), 582(19, M–2x18), 567(43, M–2x18–15), 558(4, M–60), 540(32, M–60–18), 526(31), 525(79, M–60–18–15), 522(18, M–2x18–60), 508(22), 507(53, M–60–2x18–15), 498(4, M–2x60), 483(4, M–2x60–15), 480(9, M–2x60–18), 465(22, M–2x60–18–15), 447(13, M–2x60–2x18–15), 443(6), 439(6), 423(12, M–3x60–15), 421(10), 420(13, M–3x60–18), 413(9, M–18-side chain), 411(8), 405(42, M–3x60–18–15), 399(6), 387(26, M–3x60–2x18–15), 366(6), 346(13), 323(6), 293(10), 279(7), 253(7), 239(7), 135(7), 127(8), 109(20, side chain-60–18), 43(53); pmr ($CDCl_3$, 100 MHz): δ 0.61(s, 3H), 0.96(s, 6H), 1.00(d, 3H, *J* 6.5 Hz, 21-H), 1.08(s, 3H), 1.12(s, 3H), 1.23(s, 6H, 26-H, 27-H), 2.02(s, 3H, $CH_3CO$), 2.08(s, 3H, $CH_3CO$), 2.14(s, 3H, $CH_3CO$), 2.5–2.8(br, 2H), 3.99(br d, 1H, *J* 7.5 Hz, 12β-H), 4.80(m, 1H, 24-H), 4.83(d, 1H, *J* 12.0, 3α-H), 5.16(dt, 1H, *J* 5.0 Hz, *J* 12.0 Hz, 2β-H).

2α,3β,24,25-TETRAACETATE OF **1**.—Acetylation was carried out with $Ac_2O$, AcOH and few drops of $HClO_4$. The tetracetate gave the following properties: mp 200–201° [lit. 202° (8)]; ir (KBr): ν max 3480(OH), 1740(CO) $cm^{-1}$; ms (15 eV) *m/z*(%): 660(14, $M^+$), 643(11, M–17), 642(20, M–18), 627(99, M–18–15), 600(16, M–60), 585(100, M–60–15), 582 (15, M–60–18), 567(64, M–60–18–15), 540(44, M–2x60), 525(65, M–2x60–15), 523(12), 522(18, M–2x60–18), 507(53, M–2x60–18–15), 480(7, M–3x60), 465(17, M–3x60–15), 447(15, M–3x60–15–18), 431(9, M-side chain), 425(6), 423(10), 421(8), 420(12, M–4x60), 413(8, M-side chain–18), 411(9), 405(44, M–4x60–15), 387(24, M–4x60–15–18), 346(11), 311(6), 293(9), 279(8), 269(6), 253(6), 239(8), 157(8), 127(9), 109(23, side chain–60–18), 69(7), 60(10), 55(8), 43(51), 41(12); pmr ($CDCl_3$, 100 MHz): δ 0.61(s, 3H), 0.99(s, 3H), 1.00(d, 3H, *J* 6.5 Hz, 21-H), 1.07(s, 3H), 1.11(s, 3H), 1.45(s, 3H, 26-H, 27-H), 1.50(s, 3H), 1.99(s, 3H, $CH_3CO$), 2.01(s, 3H, $CH_3CO$), 2.08(s, 3H, $CH_3CO$), 2.11(s, 3H, $CH_3CO$), 2.5(m, 2H), 4.04(d, 1H, *J* 7.0 Hz, 12β-H), 4.78(d, 1H, *J* 10.0 Hz, 3α-H), 5.16(m, 2H, 2β-H, 24-H).

2α,3β,12α,21,24R,25-HEXAHYDROXYLANOSTA-8-ENE (FASCICULOL C) (**2**).—The compound gave a violet spot by spraying the tlc plate with vanillin dissolved in sulfuric acid. It gave the following physical data: mp 242–244° (MeCN) [lit. 244° (9)]; $[\alpha]^{20}D$+73.48 (MeOH); ir (KBr): ν max 3400 (OH), 1650 (C=C), 1070, 1060, 1035 (C–O) $cm^{-1}$; ms (15 eV) *m/z*(%): 508.3833(34, $M^+$, $C_{30}H_{52}O_6$ requires 508,3764), 493(13, M–15), 490(19, M–18), 475(100, M–15–18), 472(22, M–2x18), 457(82, M–2x18–15), 454(10, M–3x18), 445(10, M–18–3x15), 439(52, M–15–3x18), 429(17, M–43–2x18), 421(19, M–15–4x18), 415(6), 413(7), 404(6), 399(7), 329(10, M–18-side chain), 315 (10), 195(14), 143(6), 129(8), 124(7), 106(16); pmr ($CD_3OD$, 100 MHz): δ 0.64 (s, 3H), 0.84(s, 3H), 1.04(s, 3H), 1.06(s, 3H), 1.07(s, 3H), 1.15(s, 3H), 1.17(s, 3H), 2.94(d, *J* 10.0 Hz, 1H, 3α–H), 3.30(m, 1H, 24–H), 3.64(m, 1H, 2β–H), 3.75 (br, 2H,$CH_2OH$), 4.03(d, 1H, *J* 10.0 Hz, 12β–H); $^{13}$C-nmr data are reported in table 1.

2α,3β,21,24-TETRAACETATE OF **2**.—The acetylation of **2** was carried out with $Ac_2O$ and pyridine at rt. The tetraacetate exhibited the following physical data: mp 94–95° [lit. 96–97° (9)]; ir (KBr): ν max 3480(OH), 1742(CO), 1650(C=C), 1250(C–O) $cm^{-1}$; ms (15 eV) *m/z*(%): 676(10, $M^+$), 658(27, M–18), 643(100, M–18–15), 640(18, M–2x18), 625(35, M–2x18–15), 616(10,

[4]*N. fasciculare* extracted with ethanol afforded together with **1** and **2**, two depsipeptide ethyl esters corresponding to a transesterification of **3** and **4** with ethanol.

TABLE 1. $^{13}$c-nmr data[+] of **1–4**.

| C n° | lano-sterol[++] | **1**($d_5$-py) | **2**($d_5$-py) | **3**($CDCl_3$) | **4**($CDCl_3$) | C°n° | lano-sterol | **1** | **2** | **3** | **4** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 35.8 t | 44.7 t | 44.4 t | 41.0 t | 41.3 t | 21 | 18.8 q | 18.0 q[e] | 61.2 t | 17.2 q[e] | 61.0 t |
| 2 | 27.9 t | 68.9 d | 68.9 d | 73.8 d | 73.8 d | 22 | 36.5 t | 34.7 t[d] | 28.1 t[b] | 33.0 t | 32.4 t |
| 3 | 79.0 d | 83.5 d | 83.5 d | 80.1 d | 80.3 d | 23 | 24.2 t | 34.2 t[d] | 33.1 t | 33.0 t | 32.4 t |
| 4 | 39.0 s | 39.7 s[a] | 39.6 s[a] | 39.4 s[a] | 39.4 s[a] | 24 | 39.6 t | 79.1 d | 78.9 d | 78.6 d | 79.0 d |
| 5 | 50.5 d | 51.0 d | 51.0 d | 50.3 d | 50.2 d | 25 | 28.1 d | 72.7 s | 72.6 s | 73.0 s | 73.0 s |
| 6 | 19.2 t | 18.7 t | 18.7 t | 18.0 t | 18.1 t | 26 | 22.6 q[i] | 26.1 q[e] | 26.0 q[e] | 26.4 q[e] | 26.4 q[e] |
| 7 | 28.3 t | 28.3 t[b] | 28.3 t[b] | 28.4 t[b] | 28.1 t[b] | 27 | 22.8 q[i] | 25.8 q[e] | 25.7 q[e] | 24.5 q[e] | 24.1 q[e] |
| 8 | 135.1 s | 134.9 s | 134.7 s | 135.3 s | 135.0 s | 28 | 24.3 q | 25.2 q[e] | 24.1 q[e] | 23.3 q[e] | 23.6 q[e] |
| 9 | 135.1 s | 133.6 s | 133.9 s | 132.0 s | 132.4 s | 29 | 28.1 q | 29.1 q[e] | 29.0 q[e] | 28.4 q[e] | 28.3 q[e] |
| 10 | 37.2 s | 38.3 s[a] | 38.4 s[a] | 38.0 s[a] | 38.1 s[a] | 30 | 15.4 q | 17.4 q[e] | 17.4 q[e] | 16.3 q[e] | 16.4 q[e] |
| 11 | 21.1 t | 26.7 t | 26.8 t | 26.4 t | 26.4 t | 1' | | | | 171.7 s[g] | 171.7 s[g] |
| 12 | 26.7 t | 72.0 d | 73.1 d | 73.1 d | 73.0 d | 2' | | | | 45.9 t[h] | 46.0 t[h] |
| 13 | 44.6 s | 49.9 s | 50.6 s[f] | 49.6 s[f] | 49.9 s | 3' | | | | 70.6 s | 70.8 s |
| 14 | 49.9 s | 49.9 s | 50.2 s[f] | 49.5 s[f] | 49.9 s | 4' | | | | 28.0 q | 28.1 q |
| 15 | 31.2 t | 32.6 t[b] | 32.3 t[b] | 31.9 t[b] | 31.7 t[b] | 5' | | | | 45.7 t[h] | 45.9 t[h] |
| 16 | 31.0 t | 29.1 t[b] | 29.7 t[b] | 29.6 t[b] | 29.6 t[b] | 6' | | | | 170.3 s[g] | 170.1 s[g] |
| 17 | 51.2 d | 43.4 d | 38.6 d | 43.2 d | 43.6 d | 7' | | | | 40.4 t | 40.0 t |
| 18 | 15.8 q | 16.7 q[c] | 17.1 q[c] | 16.6 q[c] | 16.6 q[c] | 8' | | | | 172.1 s[g] | 171.8 s[g] |
| 19 | 18.3 q | 20.4 q | 20.3 q | 19.8 q | 19.8 q | 9' | | | | 54.2 q | 54.3 q |
| 20 | 36.5 d | 36.8 d | 44.0 d | 35.9 d | 36.0 d | | | | | | |

[+]25.2 MHz, TMS. Chemical shifts in ppm. Signal multiplicity s=singlet, d=doublet, t=triplet, q=quartet obtained by "off-resonance" decoupling experiments.

[++]$^{13}$c-nmr data of lanosterol are reported from S.A. Knight, Tetrahedron Letters 83 (1973).

The chemicals shifts of the oxygenated carbons have been assigned by selective decouplings. The assignments indicated with the letters a-i can be reversed.

M–60), 598(40, M–60–18), 583(97, M–60–18–15), 580(12, M–60–2x18), 565(42, M–60–2x18–15), 556(10, M–2x60), 553(6), 541(10, M–2x60–15), 538(28, M–2x60–18), 523(63, M–2x60–18–15), 521 (11), 505(30, M–2x60–2x18–15), 496(7, M–3x60), 481(8, M–3x60–15), 478(9, M–3x60–18), 463(38, M–3x60–18–15), 445(18, M–3x60–2x18–15), 421(12, M–4x60–15), 413(9), 411(11), 403(35, M–4x60–18–15), 399(7), 385(16, M–4x60–2x18–15), 346(16), 293(9), 171(17), 159(7), 134(9), 133(9), 109(9), 95(10), 60(16), 43(68), 41(11); pmr ($CDCl_3$, 90 MHz): $\delta$ 0.62 (s, 3H), 0.94(s, 6H), 1.10(s, 3H), 1.20(s, 6H), 1.26(s, 3H), 1.99(s, 3H, $CH_3CO$), 2.06(s, 3H, $CH_3CO$), 2.10 (s, 3H, $CH_3CO$), 2.12(s, 3H, $CH_3CO$), 3.2(br, 1H, OH), 3.90(d, 1H, $J$ 7.5 Hz, 12$\beta$-H), 4.0(dd, 1H, $J_{gem}$ 1.10 Hz, $J_{vic}$ 4.0 Hz, 21–H), 4.5 (dd, $J_{gem}$ 11.0, $J_{vic}$ 2.0, 1H, 21–H), 4.82(d, 1H, $J$ 11.0, 3$\alpha$–H), 5.15(m, 1H, $J$ 11.0, $J$ 3.5 Hz, 2$\beta$–H).

2$\alpha$,3$\beta$,21,24,25-PENTAACETATE OF **2**.—The pentaacetate was isolated from both the acetylation of **2** with $Ac_2O$ and pyridine at rt and with $Ac_2O$, AcOH and $HClO_4$). It was an oil that exhibited the following data: ir (film): $\nu$ max 3480(OH), 1742(CO), 1650(C=C), 1250(C–O) $cm^{-1}$; ms(70 eV) $m/z$(%): 718(<1,$M^+$), 642(8), 640(13, M–60–18), 580(3, M–2x60–18), 411(2), 376(5), 346(7), 251(15), 248(12), 219(12), 148(26), 133(24), 125(21), 121(23), 119(21), 111(28), 109(34), 107(28), 97(38), 95(41), 81(43), 71(44), 69(56), 60(25), 57(57), 55(71), 43(100), 41(56); pmr ($CDCl_3$, 90 MHz): $\delta$ 0.63(s, 3H), 0.69(s, 6H), 1.10(s, 3H), 1.15(s, 3H), 1.24(s, 6H, 26–H, 27–H), 1.97(s, CH, $CH_3CO$), 2.00(s, 3H, $CH_3CO$), 2.03(s, 6H, $CH_3CO$), 2.10(s, 3H, $CH_3CO$), 3.74 (dd, 1H, $J$ 12.0, $J$ 6.0 Hz, 21–H), 4.05 (dd, 1H, $J$ 12.0, $J$ 4.0 Hz, 21–H), 4.74(d, 1H, $J$ 11.0 Hz, 3$\alpha$–H), 4.90(d, 1H, $J$ 7.5, 12$\beta$–H), 5.12 (dt, $J_d$ 5.0, $J_t$ 11.0 Hz, 1H, 2$\beta$–H).

2$\alpha$,3$\beta$,12$\alpha$,21,24,25-HEXAACETATE OF **2**.—The hexaacetate was prepared from acetylation of **2** with $Ac_2O$, AcOH and $HClO_4$ to gave an oil. It exhibited the following properties: ir (film): $\nu$ max 1740(CO), 1250(C–O); ms(70 eV) $m/z$(%): 760(<1, $M^+$), 700(22, M–60), 640(7, M–2x60), 625(6, M–2x60–15), 581(6), 346(10), 71(12), 69(13), 55(16), 51(9), 43(100), 41(18); pmr ($CDCl_3$, 90 MHz): $\delta$ 0.69(s, 3H), 0.89(s, 3H), 0.90(s, 3H), 1.02(s, 3H), 1.06(s, 3H), 1.40(s, 3H, 26–H or 27–H), 1.42(s, 3H, 27–H or 26–H), 1.93(s, 3H, $CH_3CO$), 1.97(s, 3H, $CH_3CO$), 2.00(s, 3H, $CH_3CO$) ,2.00(s, 3H, $CH_3CO$), 2.03(s, 6H, $CH_3CO$), 2.06(s, 3H, $CH_3CO$).

24,25-MONOACETONIDE of **2**.—The monoacetonide was prepared by the reaction of **2** with dry acetone, $CuSO_4$ and conc. $H_2SO_4$. It exhibited the following properties: mp 239–240°; ir (KBr): $\nu$ max 3450(OH) $cm^{-1}$; ms(70 eV) $m/z$(%): 548(1, $M^+$), 530(2, M–18), 516(7, M–17–15), 515(24, M–18–15), 497(7, M–2x18–15), 485(2, M–3x15–18), 479(4, M–3x18–15), 472(4, M–18–58), 457(14, M–18–58–15), 454(5, M–58–2x18), 439(24, M–58–2x18–15), 421(10, M–58–3x18–15), 197

(14), 175(10), 173(14), 161(24), 157(21), 145(24), 135(17), 133(24), 129(17), 121(24), 119(34), 109(27), 107(38), 105(34), 95(41), 93(27), 91(31), 81(38), 79(27), 71(59), 69(52), 67(34), 59(69), 58(45), 55(62), 53(17), 43(100), 41(86); pmr ($CD_3COCD_3$, $D_2O$, 90 MHz): $\delta$ 0.61(s, 3H), 0.82(s, 3H), 1.00(s, 3H), 1.02(s, 3H), 1.03(s, 3H), 1.05(s, 3H), 1.20(s, 3H), 1.26(s, 3H), 1.33(s, 3H), 2.93(d, 1H, $J$ 9.0 Hz, 3$\alpha$-H), 3.67(m, 1H, 2$\beta$-H), 3.75(br, 2H, 21-H), 4.0(d, $J$ 9.0 Hz, 1H, 12$\beta$-H).

24,25-MONOACETONIDE-2$\alpha$,3$\beta$,21-TRIACETATE OF **2**.—The compound was prepared by acetylation of monoacetonide with $Ac_2O$ and pyridine at rt. It exhibited the following properties: ir (KBr): $\nu$ max 3450(OH), 1745(C=O), 1250(C-O) $cm^{-1}$; ms (70 eV) $m/z$(%): 674(2, $M^+$), 659(2, M-15), 657(12, M-17), 642(22, M-15-17), 641(39, M-15-18), 599(9, M-15-60), 598(5, M-18-58), 584(9, M-2x15-60), 583(20, M-29-60), 581(5, M-15-18-60), 538(4, M-18-58-60), 523(17, M-18-15-58-60), 481(5), 463(12, M-18-15-58-2x60), 439(4), 421(6), 133(18), 119(20), 95(20), 83(25), 71(26), 69(24), 55(27), 43(100); pmr ($CDCl_3$, 90 MHz): $\delta$ 0.60(s, 3H), 0.90(s, 6H), 1.09(s, 9H), 1.23(s, 3H), 1.31(s, 3H), 1.39(s, 3H), 1.98(s, 3H, $CH_3CO$), 2.03(s, 3H, $CH_3CO$), 2.08(s, 3H, $CH_3CO$), 3.62(m, 1H, 24-H), 3.85(s, 1H, 12$\beta$-H), 3.99(dd, 1H, $J$ 12.0, $J$ 1.0 Hz, 21-H), 4.48(dd, 1H, $J$ 12.0, $J$ 2.0 Hz, 21-H), 4.77 (d,1H, $J$ 10.5 Hz, 3$\alpha$-H), 5.11(dt, 1H, $J_d$ 10.5, $J_t$ 4.0 Hz, 2$\beta$-H).

2,3-24,25-DIACETONIDE OF **2**.—The diacetonide had the following properties: mp 240–241° [lit. 243–244° (9)]; ms(70 eV) $m/z$(%): 588(<1, $M^-$), 570(17, M-18), 555(13, M-18-15), 552(6, M-2x 18), 537(12, M-2x18-15), 512(13, M-58-18), 494(33, M-58-2x18), 479(46, M-58-2x18-15), 454(20, M-18-2x58), 439(47, M-18-15-2x58), 521(34, M-2x18-15-2x58), 58(100), 42(81).

2$\alpha$,3$\beta$,21,24-TETRACETOXY-12$\alpha$,25-DIHYDROXYLANOSTA-7,9-DIENE.—This compound was prepared by reaction of **2** with *m*-chloroperbenzoic acid and treatment of the isolated epoxide with $H_2SO_4$. It exhibited the following data: uv(cyclohexane): $\lambda$ max 252.5, 246, 237, 207 nm; ir (KBr): $\nu$ max 3450(OH), 1740 (c=O), 1650(C=C), 1250(C-O) $cm^{-1}$; ms(70 eV) $m/z$(%): 674 (<1, $M^+$), 610(25, M-2x18-28), 609(33, M-2x18-29), 597(15), 596(33, M-18-60), 578(16, M-2x18-60), 568(11), 554(14, M-2x60), 536(22, M-18-2x60), 518(13, M-2x18-2x60), 503(11, M-15-2x18-2x60), 496(8), 475(10), 465(13), 448(18), 443(24, M-15-2x18-3x60), 441(28), 430(59), 411(54), 401(39), 388(37), 376(22), 374(19), 356(24), 157(26), 156(78), 149(32), 141(31), 139(88), 113(19), 111(54), 97(16), 85(25), 84(100), 83(22), 82(19), 75(39), 74(32), 71(33), 69(26), 57(56), 56(34), 55(36), 43(58), 42(23), 41(33); pmr ($CDCl_3$, 90 MHz): $\delta$ 0.58(s, 3H), 0.90(s, 3H), 1.00(s, 3H), 1.02(s, 3H), 1.09(s, 3H), 1.20(s, 6H), 2.01(s, 3H, $CH_3CO$), 2.02(s, 3H, $CH_3CO$), 2.03(s, 3H, $CH_3CO$), 2.10(s, 3H, $CH_3CO$), 2.39(dd, 2H, $J$ 12.0, $J$ 6.0 Hz, 1-H), 3.49(d, 1H, $J$ 5.5 Hz, 12$\beta$-H), 3.88(dd, 1H, $J$ 11.0, $J$ 7.5 Hz, 21-H), 4.23(dd, 1H, $J$ 11.0, $J$ 3.5 Hz, 21-H), 4.75(s, 1H, 24-H), 4.79(d, 1H, $J$ 10.5 Hz, 3$\alpha$-H), 5.19(dt, 1H, $J_d$ 10.5, $J_t$ 6.0 Hz, 2$\beta$-H), 5.57(d, 1H, $J$ 6.0 Hz, 7-H), 5.63(d, 1H, $J$ 5.5 Hz, 11-H).

PREPARATION OF COMPOUND **5**.—Compound **5** was prepared by oxidation of **2** with periodic acid followed by NaOH condensation. It exhibited the following properties: ir (film): $\nu$ max 3460(OH), 1720(C=O), 1690($\alpha$,$\beta$-unsaturated aldehyde) $cm^{-1}$; ms(70 eV) $m/z$(%): 428(<1, $M^-$), 410(1, M-18), 392(9, M-2x18), 377(44, M-2x18-15), 362(3, M-2x15-2x18), 349(2), 321(9), 109(20), 83(29), 58(38), 44(46), 31(100).

3$\beta$,12$\alpha$,24R,25-TETRAHYDROXYLANOSTA-8-ENE 2$\alpha$(N-GLYCYL METHYL ESTER)-3'-HYDROXY-3'-METHYLGLUTARATE (**3**).—Compound **3** gave a green spot when sprayed on the tlc plate with vanillin in sulfuric acid. It exhibited the following data: mp 94–95° [lit. 95–97° (8)]; ir (KBr): $\nu$ max 3500–3400 (OH,NH), 1740 (C=O), 1660, 1550 (CONH), 1230, 1210, 1075, 1040 (C-O) $cm^{-1}$; ms(70 eV) $m/z$ (%): 534(1, M-$C_7H_{11}NO_4$), 516(1, M-173-18), 501(4, M-173-18-15), 483(6, M-173-2x18-15), 459(9, M-$C_5H_{13}NO_5$-18-15), 441(22, M-215-2x18-15), 423(11, M-215-3x18-15), 405(5, M-215-4x18-15), 376(3), 329(2), 251(7), 127(10), 121(9), 119(11), 114(21), 109(19), 107(10), 105(10), 95(23), 93(13), 85(13), 81(31), 69(31), 67(32), 59(29), 57(22), 55(44), 43(100), 41(35); pmr ($CD_3COCD_3$, 100 MHz): $\delta$ 0.64(s, 3H), 0.88(s, 3H), 1.06(s, 3H), 1.12(s, 9H), 1.28(s, 3H), 1.36(s, 3H), 1.9–2.2(m, 4H, $CH_2$), 2.54 and 2.62(AB system, 2H, $J_{AB}$ 14.5 Hz, $CH_2$), 2.57 and 2.70(AB system, 2H, $J_{AB}$ 13.5 Hz, $CH_2$), 3.1–3.4(m, 3H, 2 C*H*OH, OH), 3.70 (s,3H, $CH_3O$), 4.04(d, 2H, $J$ 6.0 Hz, C$H_2$NH), 5.03(m, 1H, $J$ 11.0 Hz, $J$ 4.0 Hz, 2$\beta$-H), 7.78(t, 1H, $J$ 6.0 Hz, NH).

3$\beta$,12$\alpha$,21,24R,25-PENTAHYDROXYLANOSTA-8-ENE 2$\alpha$(N-GLYCYL METHYL ESTER)-3'-HYDROXY-3'-METHYLGLUTARATE (**4**).—It gave a violet spot when sprayed on the tlc plate with vanillin in sulfuric acid. It gave the following data: mp 103–105° [lit. 102–103° (9)]; ir (KBr): $\nu$ max 3550–3300 (OH, NH), 1740 (C=O), 1660, 1550 (CONH), 1200, 1065, 1040 (C-O) $cm^{-1}$; ms, (15 eV) $m/z$(%): 723 ($M^+$ detected by field desorption), 550 (10, M-$C_7H_{11}NO_4$), 532 (16, M-173-18), 517(34, M-173-18-15), 508(30, M-$C_5H_{13}NO_5$), 499(28, M-173-2x18-15), 493 (10, M-215-15), 490 (22, M-215-18), 481(9, M-173-3x18-15), 475(100, M-215-18-15), 472(16, M-215-2x18), 457(96, M-215-2x18-15), 454(12, M-215-3x18), 439(76, M-215-3x18-15), 421(50, M-215-4x18-15), 413(12), 403(14, M-215-5x18-15), 329(14), 315(16), 195(28), 159(14), 145(10), 143(12), 129(32), 105(18), 95(16), 91(16), 79(12), 71(18), 67(13), 55(20), 43(51), 41(28); pmr($CD_3COCD_3$, 100 MHz): $\delta$ 0.63(s, 3H), 0.89(s, 3H), 1.08(s, 3H), 1.10(s, 3H), 1.13(s, 9H), 1.39(s, 3H), 2.0–2.2(m, 4H, $CH_2$), 2.55 and 2.64(AB system, 2H, $J_{AB}$ 14.5 Hz, $CH_2$), 2.60 and 2.71 (AB system, 2H, $J_{AB}$ 13.5 Hz,

$CH_2$), 3.24 (d, 1H, $J$ 10.0 Hz, 3$\alpha$H), 3.32 (m, 1H, 24-H), 3.70 (s,3H, $CH_3O$), 3.79(br, 2H, 21-H), 4.00(d, 2H, $J$ 6.0 Hz, $CH_2NH$), 5.02(m, 1H, 2$\beta$-H), 7.77 (t, 1H, $J$ 6.0 Hz, $NH$-$CH_2$).

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