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METABOLITES OF BIRD'S NEST FUNGI—19†

NEW TRITERPENOID CARBOXYLIC ACIDS FROM *CYATHUS STRIATUS* AND *CYATHUS PYGMAEUS*

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Abstract—The bird's nest fungus *Cyathus striatus* Willd. ex. Pers. yielded the known triterpenes glochidone (**1**), glochidonol (**2**), glochidiol (**3**) and glochidiol diacetate (**4**) as well as four new triterpenoic acids, cyathic acid (**5**), striatic acid (**6**), cyathadonic acid (**7**) and epistriatic acid (**8**). Another species, *Cyathus pygmaeus* Lloyd, contained cyathic acid (**5**) and an additional new compound, pygmaeic acid (**9**). The structural assignments are based on spectroscopic data and chemical correlations.

Of the many species of bird's nest fungus (family Nidulariaceae) found worldwide there are only a few common to North America. One is the species *Cyathus striatus*. We have recently examined this fungus as part of an investigation of the metabolites of bird's nest fungi.[2]

A preliminary investigation by others[3] had reported the presence of unidentified bacteriostatic compounds in *Cyathus striatus*. Subsequently Anke and coworkers identified a group of C_{25} compounds called the striatins from the mycelia of *Cyathus striatus*.[4]

Cyathus striatus was grown in liquid culture for 25 days, the mycelia harvested and extracted with hot MeOH. After concentration and removal of the crystalline sugars, the MeOH extract was chromatographed on Sephadex LH20. This yielded the previously identified triterpenes glochidone (**1**), glochidonol (**2**), glochidiol (**3**), glochidiol diacetate (**4**)[5] and a new triterpene acid, for which we suggest the name cyathic acid (**5**). Investigation of minor chromatographic fractions yielded three congeners of cyathic acid: striatic acid (**6**), cyathadonic acid (**7**), and epistriatic acid (**8**).

A similar investigation of the mycelial extracts of *Cyathus pygmaeus* yielded cyathic acid (**5**) and an additional new compound, pygmaeic acid (**9**).

Cyathic acid has the molecular formula $C_{34}H_{52}O_6$ as determined by high resolution mass spectroscopy (HRMS) and microanalysis. The IR spectrum of cyathic acid (**5**) shows carbonyl absorption at 1740, 1720 and 1685 cm^{-1} and strong absorption at 1250 and 1240 cm^{-1}. The ^1H NMR spectrum reveals the presence of two acetoxyl groups. The remaining two oxygens are present as part of a carboxyl group as confirmed by the formation (CH_2N_2) of a methyl ester (**10**) and by the lack of ketone adsorption in the UV spectrum. Cyathic acid is not soluble in aqueous Na_2CO_3 and is less polar than glochidone (**1**) on TLC (Si gel).

The functionality of cyathic acid was elucidated in the following way. Methyl cyathate (**10**) shows ester absorption at 1719 cm^{-1} and no IR absorption at 1685 cm^{-1}. Treatment of ester **10** with $LiAlH_4$ in ether or tetrahydrofuran (THF) gave diol **11**. The IR spectrum of **11** no longer shows the acetate absorption but the ester carbonyl at 1719 cm^{-1} remains intact. Compound **11** was converted to a dimethyl ether (**12**) on treatment with methyl iodide/sodium hydride. Direct reduction of cyathic acid (**5**) with $LiAlH_4$ in ether afforded a dihydroxy acid **13** which on Jones' oxidation afforded the diketone **14**. Hydrogenation of cyathic acid (PtO_2/MeOH) afforded a dihydro derivative **15**. These observations serve to establish the nature of the functional groups in cyathic acid, i.e. a hindered carboxyl group, two acetoxyl groups and a double bond.

The stereochemical relationship of these groups and the nature of the carbon skeleton of cyathic acid was elucidated as follows. The ^1H NMR spectrum of cyathic acid **5** clearly shows the presence of eight methyl groups. Two of these (δ2.0 and δ1.97) are due to acetyl functions as evidenced by their absence in the diol **13**. An isopropenyl group is indicated by a vinylic methylene ($\approx \delta$4.6) and a vinylic methyl group (δ1.67). Catalytic hydrogenation leads to the formation of an isopropyl group (δ0.85) in **15**. The remaining five methyl groups are all singlets indicating they are attached to quaternary centers. This evidence is sufficient to suggest that cyathic acid is a pentacyclic triterpene of either the lupane, hopane, or fernane type, providing that one assumes that one of the methyl groups of these types is present as a carboxyl group. In the dihydro derivative **15** of cyathic acid the absence of the vinylic methylene group permits the clear observation in the ^1H NMR signals of the methine protons on the carbons bearing the acetoxyl functions. These protons appear as double doublets with coupling constants of 11 and 5 Hz and 12 and 4.5 Hz, respectively, indicating that the acetoxyl functions are equatorial provided they

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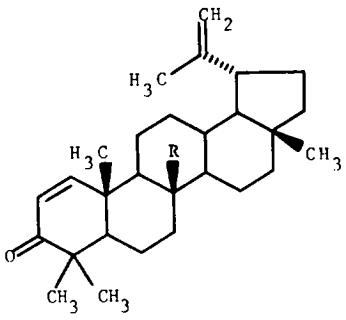
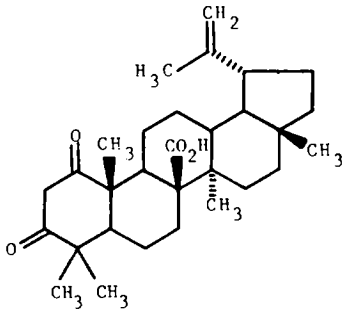


1 R = CH_3

7 R = CO_2H

27 R = CO_2CH_3



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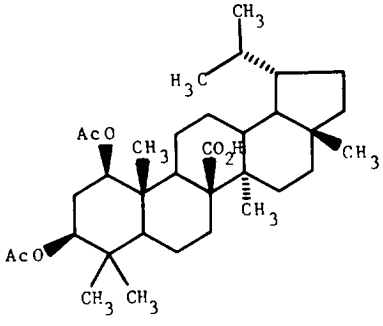

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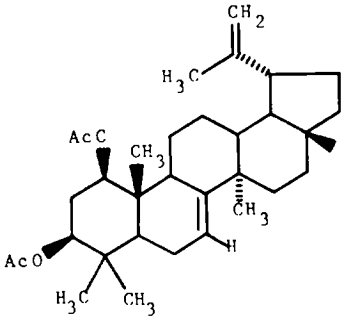
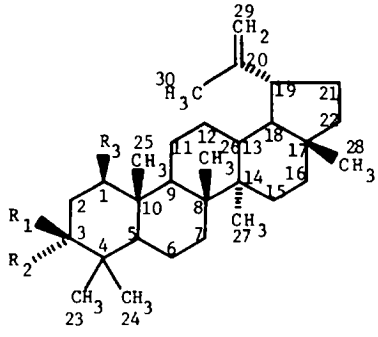


16

are located on six membered rings. In the dimethyl derivative of the ester **12** a similar pair of double doublets is observed in its ^1H NMR with coupling constants of 11 and 5 Hz and 12 and 4 Hz. Methyl cyathate (**10**) clearly shows the presence of an additional methyl group at δ3.7 in the ^1H NMR spectrum in keeping with the formation of a methyl ester. Because of the co-occurance of glochidone (**1**), glochidonol (**2**), glochidiol (**3**) and its diacetate **4** with cyathic acid (**5**) in *C. striatus*, a lupene skeleton was assumed as a working hypothesis.

The high resolution mass spectrum of cyathic acid



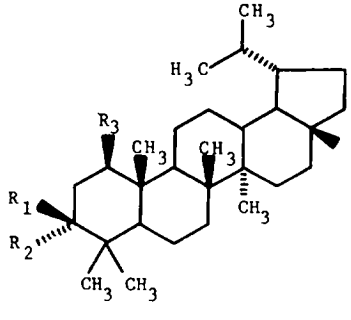
2 R_1 = R_2 = O R_3 = OH

3 R_1 = OH R_2 = H R_3 = OH

4 R_1 = OAc R_2 = H R_3 = OAc

17 R_1 = R_2 = O R_3 = OAc

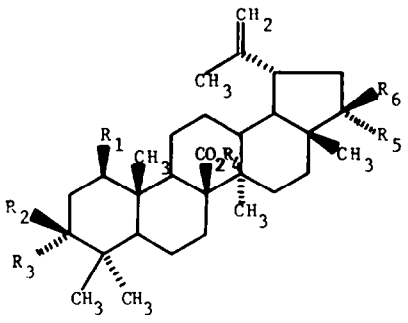
18 R_1 = OH R_2 = H R_3 = OAc



19 R_1 = R_2 = O R_3 = H

20 R_1 = OH R_2 = H R_3 = H

21 R_1 = OAc R_2 = H R_3 = H



5 R_1 = OAc R_2 = OAc R_3 = H R_4 = H R_5 = H R_6 = H

6 R_1 = OAc R_2 = OH R_3 = H R_4 = H R_5 = H R_6 = H

8 R_1 = OAc R_2 = H R_3 = OH R_4 = H R_5 = H R_6 = H

9 R_1 = OAc R_2 = OAc R_3 = H R_4 = H R_5 = OH R_6 = H

10 R_1 = OAc R_2 = OAc R_3 = H R_4 = CH_3 R_5 = H R_6 = H

11 R_1 = OH R_2 = OH R_3 = H R_4 = CH_3 R_5 = H R_6 = H

12 R_1 = OCH_3 R_2 = OCH_3 R_3 = H R_4 = CH_3 R_5 = H R_6 = H

13 R_1 = OH R_2 = OH R_3 = H R_4 = H R_5 = H R_6 = H

22 R_1 = OAc R_2 = OH R_3 = H R_4 = CH_3 R_5 = H R_6 = H

23 R_1 = OH R_2 = OAc R_3 = H R_4 = CH_3 R_5 = H R_6 = H

24 R_1 = OAc R_2 = H R_3 = OH R_4 = CH_3 R_5 = H R_6 = H

25 R_1 = OAc R_2 = R_3 = O R_4 = CH_3 R_5 = H R_6 = H

26 R_1 = OAc R_2 = H R_3 = OAc R_4 = CH_3 R_5 = H R_6 = H

28 R_1 = OAc R_2 = OAc R_3 = H R_4 = H R_5 = R_6 = O

29 R_1 = OAc R_2 = OAc R_3 = H R_4 = CH_3 R_5 = OH R_6 = H

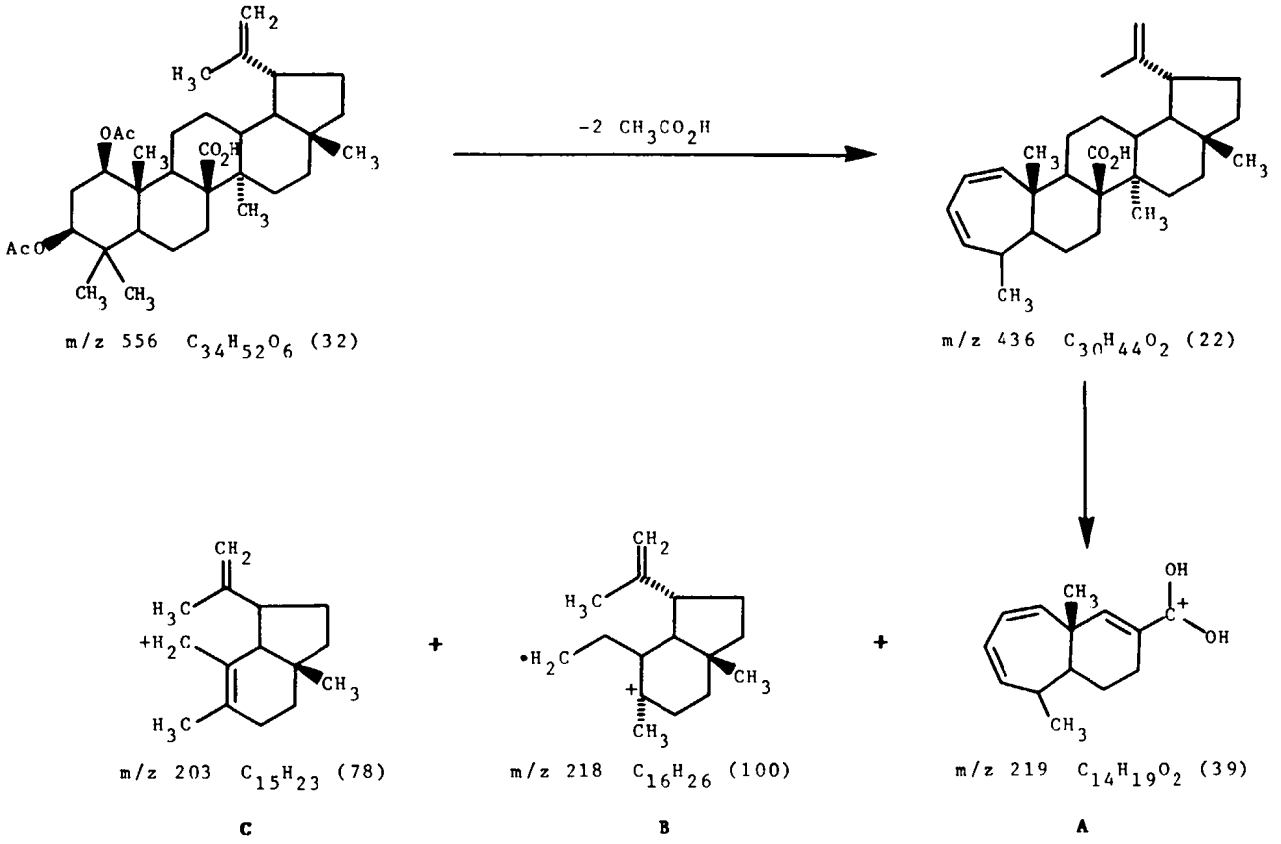
30 R_1 = OAc R_2 = OAc R_3 = H R_4 = CH_3 R_5 = OAc R_6 = H

31 R_1 = OAc R_2 = OAc R_3 = H R_4 = CH_3 R_5 = OMs R_6 = H

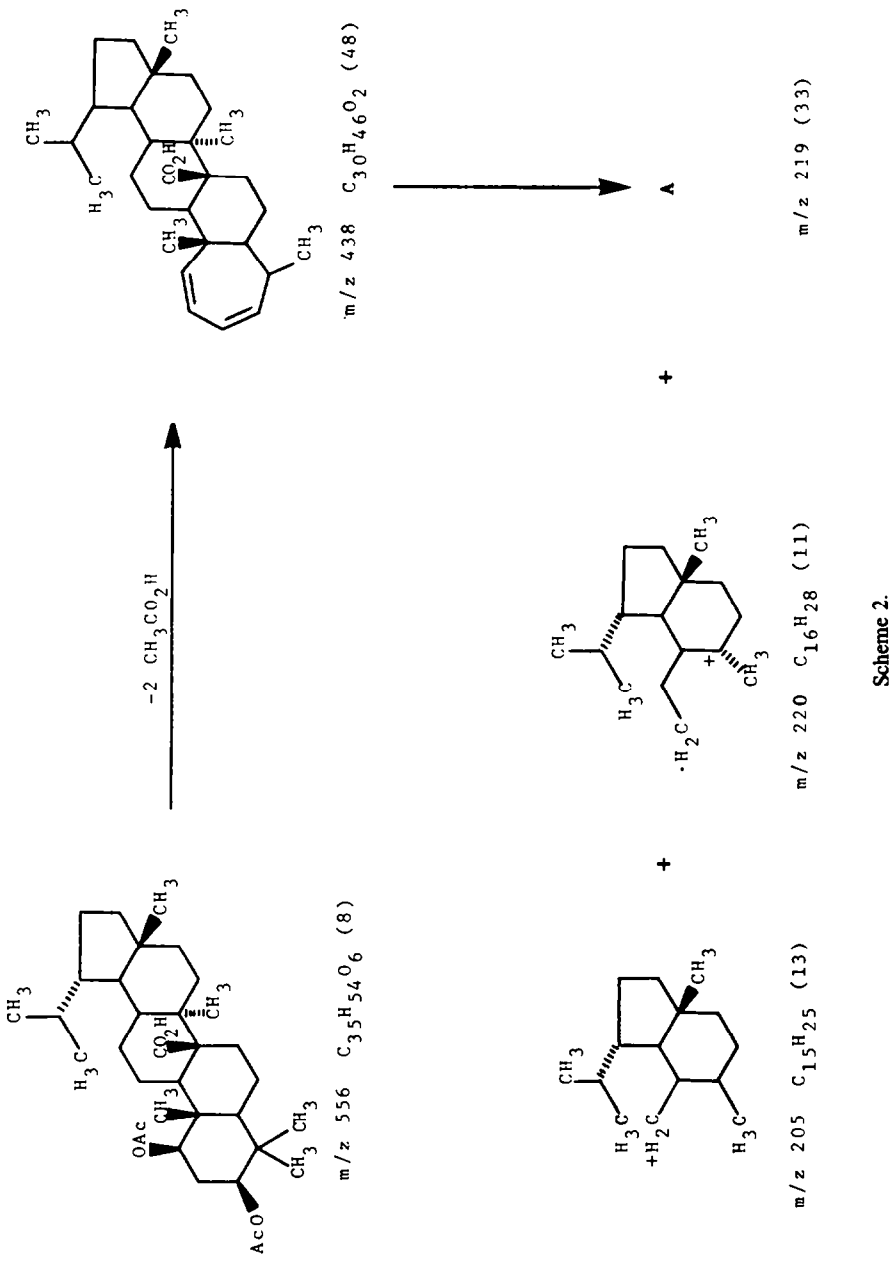
5 is informative (Scheme 1). The molecular ion loses two molecules of acetic acid to give the prominent ion at *m/z* 436. This ion is the precursor for ions at *m/z* 219 ($C_{14}H_{19}O_2$) and *m/z* 218 ($C_{16}H_{26}$). From the molecular formulas of these ions it is clear that they represent two distinct portions of the parent molecule. The fragment at *m/z* 218, which is the base peak, cannot be derived from that part of the molecule which contains the acetoxyl groups. The highly unsaturated fragment at *m/z* 219 not only contains the carboxyl group but also must include the original site of the acetoxyl functions. The presence of a substantial fragment at *m/z* 203 ($C_{15}H_{23}$) is common with many types of pentacyclic triterpenes. This ion is found in lupane triterpenes which bear no oxygen functions in the D and E rings.[6]

Further evidence that the ions at *m/z* 218 and *m/z* 219 represent the CDE and AB rings respectively, of a lupane triterpene is as follows. In dihydrocyathic acid (**15**) (Scheme 2) the fragment at *m/z* 219 (i.e. from the AB rings) appears unchanged but the fragments originally found in **5** at *m/z* 218 and *m/z* 203 now appear at *m/z* 220 and *m/z* 205. A fragment at *m/z* 395 in **15** corresponds to the loss of the isopropyl group, an event not observed in **5** as this group is present as an isopropenyl function. In methyl cyathate (**10**) the fragment at *m/z* 218 representing the CDE rings is unchanged when compared to the free acid **5** whereas a new ion at *m/z* 233 ($C_{15}H_{21}O_2$) represents an augmented 219 fragment. In the mass spectrum of the dimethoxy ester **12** (Scheme 3) one observes for the first time a completely intact AB ring fragment at *m/z* 297 ($C_{17}H_{26}O_4$). This fragment occurs because of the lesser tendency of methoxyl groups to be eliminated and confirms that all the oxygen containing functional groups of cyathic acid are located on the AB ring portion. Since we assume that the carboxyl group is derived from one of the lupane methyl groups, it can only be located at C-4, C-8 or C-10.

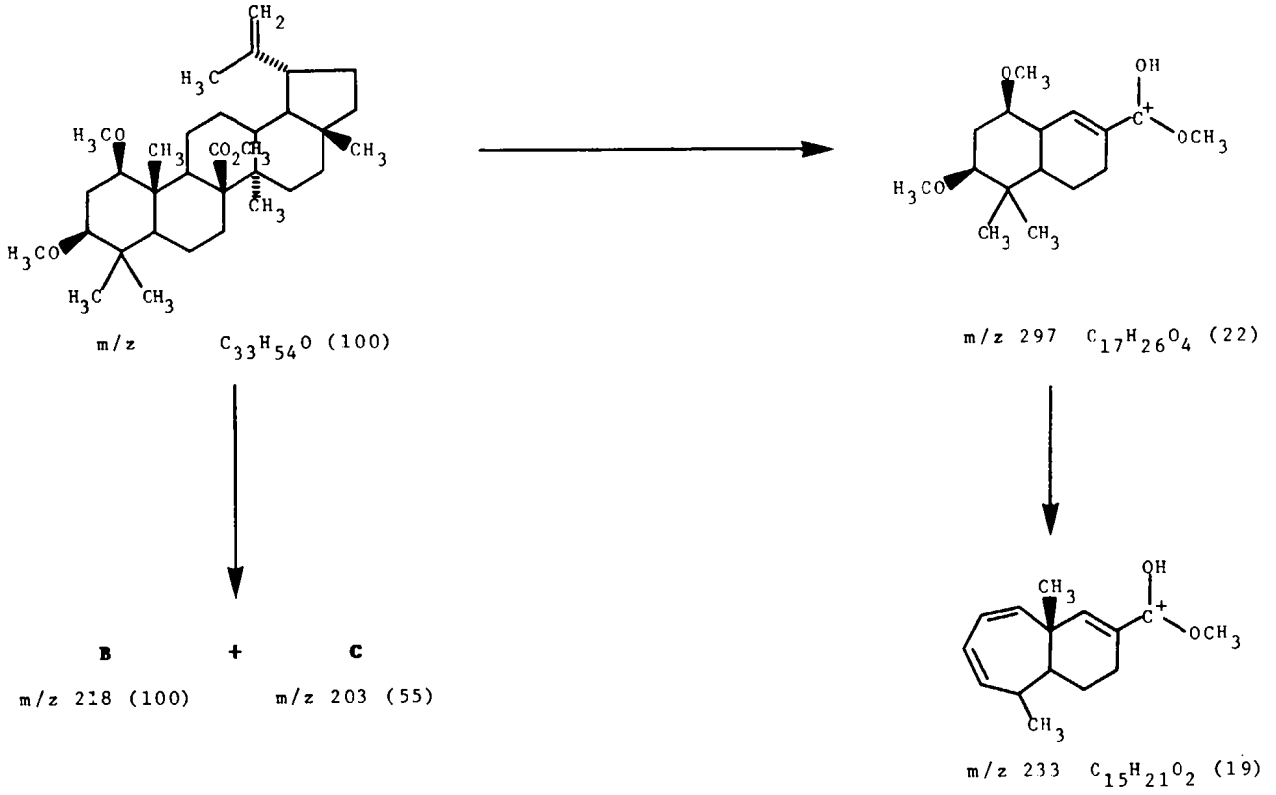
Oxidation of diol **13** with Jones' reagent gives the enolized 1,3-diketone **14** ($\lambda_{max} = 257$ ($\epsilon = 10{,}000$),


-2 CH3CO2H
m/z 556 C34H52O6 (32)
m/z 436 C30H44O2 (22)
m/z 203 C15H23 (78)
m/z 218 C16H26 (100)
m/z 219 C14H19O2 (39)
C
B
A

Scheme 1.


-2 CH3CO2H
m/z 556 C35H54O6 (8)
m/z 438 C30H46O2 (48)
A
m/z 205 C15H25 (13)
m/z 220 C16H28 (11)
m/z 219 (33)

Scheme 2.


m/z C33H54O (100)
m/z 297 C17H26O4 (22)
B + C
m/z 218 (100)
m/z 203 (55)
m/z 233 C15H21O2 (19)

Scheme 3.

$\lambda_{max}^{-OH} = 288$ ($\epsilon = 26{,}000$). This 1,3-relationship limits the placement of the hydroxyl functions of **11** and thus the acetoxyl functions of **5** to the A ring.

The carboxyl group in **5** is extraordinarily hindered. It does not react with diborane or with $LiAlH_4$ even under forcing conditions. It is also slow to react with CH_2N_2 requiring 6–12 hr for esterification to be complete. Methyl cyathate (**10**) resisted all attempts to reduce the carboxylate function. Treatment with a large excess of $LiAlH_4$ in refluxing THF for 72 hr gave a quantitative yield of the diol **11** with no trace of triol. Decarboxylation of cyathic acid with $Pb(OAc)_4$ gives the diacetoxy olefin **16** in quantitative yield. The ^1H NMR of **16** clearly shows a single new vinyl proton at $\delta 5.44$ (i.e. a trisubstituted double bond). The mass spectral evidence clearly indicates that the carboxyl group is associated with the AB ring system. Since oxidative decarboxylation gives rise to a trisubstituted double bond, the carboxyl group must be at C-8. (Since the fernane family lacks a methyl group at C-8, it can be excluded from further consideration.) The hindered nature of the carboxyl group in cyathic acid is consistent with its assignment to C-8 since a study of Dreiding models of **5** shows that the C-26 carbon is subject to steric hindrance from the methyl groups at C-10 and C-4 and from axial hydrogens at C-6, C-11 and C-13.

Our lack of success in reducing the carboxyl function at C-8 to a methyl group, precluded any chemical correlation of cyathic acid (**5**) with that of a known compound. Further evidence for the lupane skeleton of **5** was provided by a detailed ^{13}C NMR analysis of cyathic acid derivatives and various known lupanes. In order to provide lupane-type model compounds with functionality in ring A similar to that of cyathic acid, glochidonol **2** was acetylated to give the acetate **17**.[5] The carbonyl in **17** was reduced with $NaBH_4$ to form **18** which was acetylated to give the diacetate **4**.[5] Glochidone **1** was hydrogenated using Pt_2O/H_2 to give the lupanone **19**.[7] Compound **19** was reduced to lupanol **20** using $NaBH_4$ and the latter acetylated to give lupanol acetate **21**.[7] Table 1 provides the ^{13}C NMR chemical shifts assignment for these known lupane derivatives.

The various carbons in the lupane skeleton were assigned in the following manner. Carbons 1 to 6

Table 1. ^{13}C Chemical shifts of some lupane derivatives

Carbons	Compounds a							
	1	19	20	21	2	17	18	4
1	159.6	39.6	38.8	38.4	79.6	80.4	80.9	80.3
2	125.1	33.6	27.4	23.7	45.2	41.8	33.4	29.9
3	205.2	217.7	79.0	81.0	216.1	215.2	75.0	78.4
4	44.6	47.3	38.9	37.8	47.1	46.8	38.7	37.8
5	53.4	54.9	55.3	55.4	51.4	50.8	53.2	53.0
6	19.2	19.7	18.4	18.2	19.6	19.5	17.8	17.7
7	33.7	34.1	34.4	34.3	33.0	32.7	34.0	34.0
8	41.7	40.8	40.9	40.9	41.2	41.0	41.3	41.3
9	44.4	49.4	50.2	50.0	50.8	50.3	51.0	51.0
10	39.5	36.8	37.2	37.1	43.0	41.9	42.8	42.2
11	21.2	21.5	21.0	21.0	23.0	22.4	22.9	22.8
12	25.0	26.8	26.8	26.8	25.2	25.2	25.0	25.0
13	38.2	37.9	37.8	37.8	38.0	38.0	37.4	37.4
14	42.7	43.1	43.2	43.2	43.0	42.9	42.9	42.9
15	27.3	27.3	27.4	27.4	27.5	27.5	27.5	27.5
16	35.4	35.5	35.6	35.6	35.6	35.5	35.6	35.6
17	43.0	43.1	43.0	43.0	43.0	42.9	42.9	42.9
18	48.1	47.6	47.6	47.6	48.3	48.1	48.2	48.2
19	47.8	44.7	44.7	44.7	48.0	48.0	48.0	48.0
20	150.5	29.4	29.4	29.6	150.7	150.5	150.6	150.6
21	29.8	21.9	21.9	21.9	29.8	29.8	29.9	29.9
22	39.9	39.6	40.4	40.4	40.4	39.9	39.8	40.0
23	27.8	26.7	28.0	28.0	28.0	28.6	27.8	27.8
24	21.4	21.0	15.4	16.6	19.9	19.7	14.9	16.0
25	19.0	15.9	16.0	16.0	11.86	12.8	13.2	13.2
26	16.4	15.8	16.1	16.1	16.0	15.8	16.2	16.1
27	14.4	14.4	14.5	14.4	14.5	14.4	14.4	14.4
28	18.0	18.1	18.1	18.1	18.1	18.0	18.0	18.0
29	109.5	15.2	15.2	15.2	109.5	109.6	109.5	109.5
30	19.3	23.0	23.0	23.0	19.3	19.3	19.2	19.2
C_1-$\underline{C}H_3CO$						21.6	21.9	21.8
C_1-$CH_3\underline{C}O$						170.3	70.5	170.2
C_3-$\underline{C}H_3CO$				21.3				21.1
C_3-$CH_3\underline{C}O$				170.9				182.5

and 9 to 11 were readily assigned on the basis of the shifts induced by the various functional group modifications in the A ring, e.g. the presence of a ketone at C-3 causes a well documented effect at C-4, C-5, and C-6.[8] Similarly a substituent at C-1 causes a profound effect at carbons 9, 10 and 11. C-7 and C-8 remain relatively unaffected by changes of functionality in the A ring and ^{13}C assignments are based on comparison with reported values.[8] Carbons 20, 29 and 30 showed large changes whereas carbon 12 was recognized by the small downfield shift induced by hydrogenation of the isopropylidene double bond (**19** compared with **1**). Carbons 13, 14, 15 and 16 were assigned by comparison with the known literature values. The only remaining aliphatic singlet is assigned to C-17. C-18 and C-19 are both doublets and can be distinguished from one another since C-19 is shifted upfield by hydrogenation of the isopropylidene double bond (**19** compared with **1**). In a similar fashion C-21 (triplet) can be distinguished from C-22 (triplet, **19** compared with **1**).

The methyl signals were assigned in the following way. The three A ring methyl groups (C-23, C-24, C-25) are readily determined as a result of observed substituent shifts from changes in functionality. Carbons 26 and 28 are assigned because they display the largest and smallest couplings respectively in the off-resonance mode. The remaining aliphatic methyl signal is thus assigned to C-27. Table 1 establishes the ^{13}C chemical shifts for various lupane derivatives which serves as a data base for study of the cyathic acid skeleton. Interestingly our values for lupanol **20** are in good agreement with those reported by Wenkert *et al.*[8]

Table 2 lists the ^{13}C chemical shifts of three naturally-occurring cyathic acids and four cyathic

Table 2. ^{13}C Chemical shifts of cyathic acid derivatives

Carbons	Compounds 5	10	22	11	8	27	9	28
1	78.1[A]	78.0[A]	79.0	75.8[A]	77.4[A]	157.8	78.1[A]	78.1[A]
2	30.0	30.0	32.8	37.0	32.2	126.0	30.1	30.1
3	76.3[A]	76.2[A]	74.4	74.7[A]	76.1[A]	-[D]	76.2[A]	76.6[A]
4	37.7	37.7	38.6	38.3	37.4	44.5	37.9	37.9
5	53.8	53.8[C]	53.8	53.6	48.7	53.8	53.8	53.8
6	19.6	19.6	19.3	19.4[D]	20.1	20.6	19.4	19.4
7	30.2	30.4	30.3	30.5	30.6	30.5	30.2	29.9
8	53.9	53.8	53.8	53.6	54.0	54.4	53.6	53.7
9	52.4	52.4	52.5	53.0	52.5	47.1	52.6	52.6
10	42.7[B]	42.5[B]	42.5[B]	43.8	42.7	40.6	42.6	42.8
11	23.4	23.6	23.6	24.2	23.7	21.0	23.5	23.3
12	24.3	24.3	24.2	24.2	24.6	24.3	24.3	23.9
13	36.5	36.7	36.7	36.9	36.9	37.7	36.4	36.5
14	42.9[B]	42.9[B]	42.8[B]	42.5[C]	43.3	43.0	43.0	43.1
15	30.7	30.7	30.6	30.5	30.8	30.6	30.3	30.3
16	35.2	35.2	35.0	34.9	35.5	35.2	28.1	28.1
17	42.9[B]	42.7[B]	42.7[B]	42.1[C]	43.2	42.8	47.4	50.1
18	48.4	48.4	48.2	48.5	48.8	48.4	42.8	44.2
19	47.8	47.8	47.6	47.9	48.0	47.7	47.6	45.8
20	150.4	150.4	150.5	150.1	150.8	150.6	149.6	146.9
21	29.8	30.0	29.8	29.5	30.2	29.9	41.1	42.6
22	39.6	39.6	39.4	39.2	39.9	39.7	78.4	218.2
23	27.8	27.8	27.5	27.2	27.6	27.5	27.9	27.8
24	15.9	15.9	14.6	14.2	11.8	21.5	16.0	16.0
25	11.9	11.9	11.6	10.0	11.8	18.5	12.0	11.9
26	170.5	175.9	176.3	175.8	178.5	176.2	180.8	170.0
27	16.1	16.0	15.7	15.2	15.9	15.5	15.9	15.6
28	17.7	17.8	17.6	17.0	17.9	17.9	17.5	14.3
29	109.4	109.6	109.3	108.8	109.6	109.6	110.5	112.5
30	19.3	19.3[D]	19.3	19.2	19.5	19.4	19.0	18.8
C_1-$\underline{CH_3}CO$	21.7	21.7	21.6	-	21.9	-		21.7
C_1-$CH_3\underline{CO}$	170.5	170.2	171.2	-	170.6	-		170.2
C_3-$\underline{CH_3}CO$	21.0	21.0	-	-	-	-		21.0
C_3-$CH_3\underline{CO}$	181.3	181.4	-	-	-	-		180.9
CO_2-CH_3		50.9	50.7	49.9	-	51.0		-

[A] Carbons 1 and 3 may be interchanged.

[B] Carbons 10, 14 and 17 may be interchanged.

[C] Carbons 14 and 17 may be interchanged.

[D] No found.

Table 3. ^{13}C Chemical shift perturbations induced in the lupane skeleton by C-8 COOR

	4 → 10	18 → 22	1 → 27
C-1	-1.9	-1.9	-1.8
C-3	-2.2	-	-
C-6	+2.1	+1.5	+1.4
C-7	-3.6	-3.7	-3.2
C-8	+12.5	+12.5	+12.7
C-9	+1.4	+1.5	+2.7
C-15	+3.1	+3.1	+3.3
C-25	-1.6	-1.6	.5
C-26	+161.5	+161.9	+161.8

acid derivatives. The chemical shift assignments for these compounds are based on the chemical shift values listed in Table 1. A rational correlation between the cyathic acid series (Table 2) and the lupane series (Table 1) is possible. Carbon assignments for 22 of the 30 carbons agree within 1 ppm. Only eight carbons show perturbations greater than 1 ppm as would be expected from the introduction of the carboxyl group at C-8 and furthermore the preturbations agree with literature values.[9,10]

The perturbations induced by the presence of the C-8 carboxyl in the lupane skeleton can be observed by comparison of three different cyathic acid-lupane pairs: the 1,3-diacetate pair **10** and **4**; the 1-acetoxy-3-hydroxy pair **22** and **18**; and the α,β-unsaturated ketone pair **1** and **7**. These data are presented in Table 3. Although the functionality of these pairs is quite different, the observed perturbations at C-8 and C-26 are very similar and of anticipated magnitude. Conformational changes induced in the lupane skeleton by the introduction of the C-8 carboxyl group can account for the remaining 6 perturbations and the magnitude of these perturbations is self-consistent for each carbon between pairs. Such a similarity would not be possible if cyathic acid (**5**) did not possess a lupane skeleton.

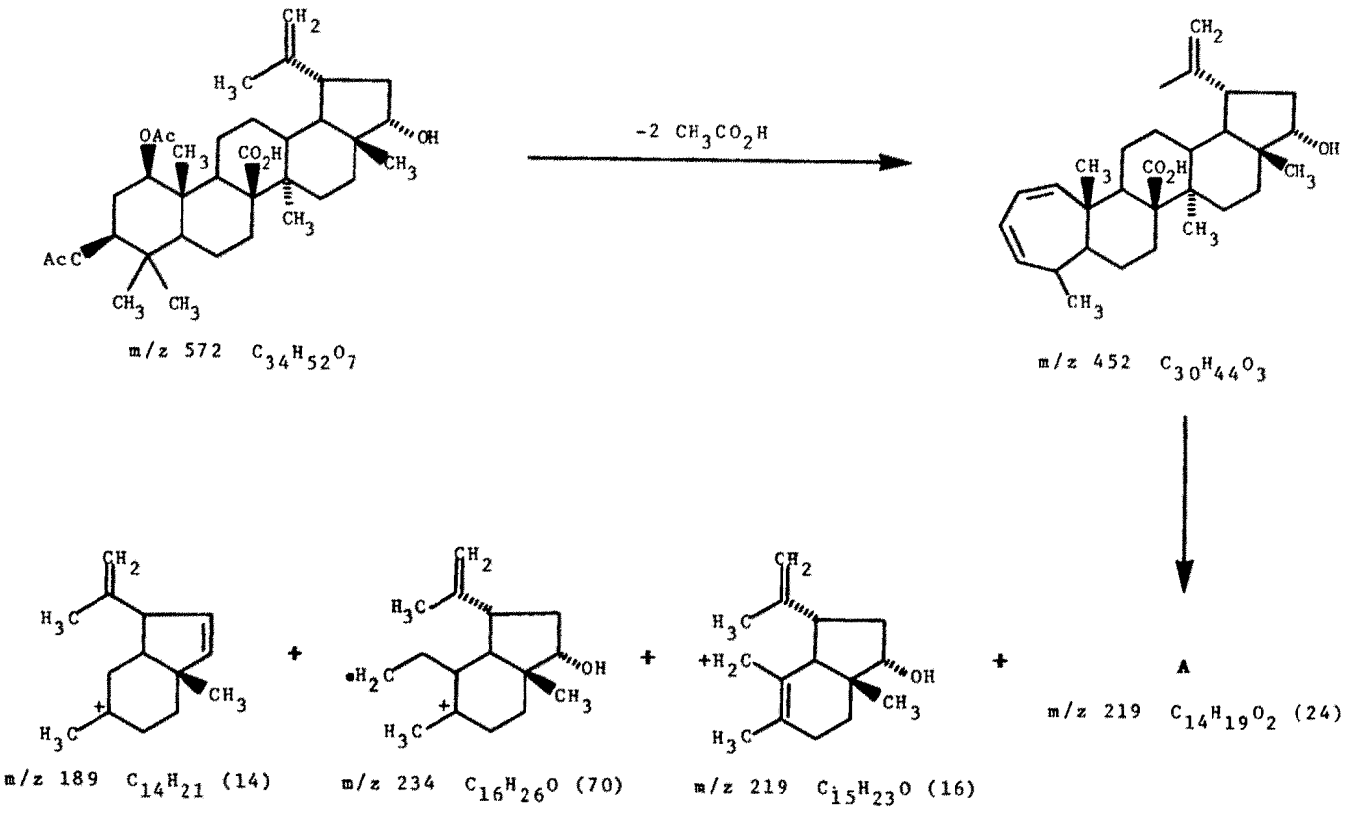
Striatic acid, assigned structure **6**, has the molecular formula $C_{32}H_{50}O_5$ (HRMS). The ^1H NMR spectrum shows the presence of an acetoxy methine proton at δ4.40 and an hydroxy methine proton at δ3.26. The IR spectrum displays carbonyl absorbption at 1740 and 1680 cm^{-1}. Treatment of striatic acid with diazomethane gives after 6 hr a quantitative yield of the methyl ester **22**. This same compound was obtained from methyl cyathate (**18**) by partial hydrolysis using Na_2CO_3 in MeOH. Such a reaction would be expected to produce the 3-hydroxy compound **22** rather than the 1-hydroxy compound **23** since it is well established that substituents at C-1 in triterpenes (steroids) are sterically hindered due to the proximity of the C-11 hydrogens.

Addition of pyridine-d_5 to a $CDCl_3$ solution of **22** induces a significant shift of two methyl signals (C-4 methyls) in its ^1H NMR. This, coupled with the presence of an hydroxy methine proton at δ3.26 (d of d, J = 4.5 and 12 Hz), clearly indicates an equatorial hydroxyl group at C-3.[11] Acetylation of striatic acid (**6**) (Ac_2O/pyridine) gives cyathic acid (**5**) as the sole product.

Epistriatic acid (**8**) appeared to be very similar to **6**. The HRMS ($M^+ = C_{32}H_{50}O_5$) and IR spectrum are very nearly identical with those of **6**. However in the ^1H NMR spectrum an equatorial hydroxy methine proton appears at δ3.51 (d of d, J = 2.5 and 3.5 Hz) along with an axial acetoxy methine proton at δ4.90 (d of d, J = 9.5 and 5.5 Hz). In the ^1H NMR of **8** only one methyl group (C-10) and the acetoxy proton at C-1 show a downfield shift on addition of pyridine-d_5. This is in agreement with the assignment of the hydroxyl group to the 3α position,[10] therefore compound **8** is epimeric with **6** at C-3. Treatment of **8** with CH_2N_2 gave the ester **24**. Oxidation of ester **22** or ester **24** with Jones' reagent in acetone gave in both cases the ketone **25**. On acetylation, **24** gave a diacetate **26** which differed from methyl cyathate **10** by ^1H NMR and by TLC.

Cyathadonic acid (**7**) has the molecular formula $C_{30}H_{44}O_3$ (HRMS). The ^1H NMR spectrum shows vinylic protons at δ7.19 (d, J = 10 Hz) and δ5.85 (d, J = 10 Hz). This, coupled with the UV spectrum (MeOH λ_{max} at 230, ϵ = 6400), suggests that **7** is an α,β-unsaturated ketone, a fact corroborated by a strong IR absorption at 1676 cm^{-1}. Compound **7** reacts slowly with diazomethane to give the methyl ester **27**. The ester **27** is identical with the product obtained by treatment of the ketone **25** with NaOH in MeOH. Thus cyathadonic acid (**7**) is the cyathic acid analogue of glochidone.

Finally, a cyathic acid derivative containing oxygen functionality in the E ring was isolated from *C. pygmaeus*. Pygmaeic acid (**9**) has the molecular formula $C_{34}H_{52}O_7$ (HRMS). The IR spectrum shows broad absorption at 3400 cm^{-1} as well as carbonyl absorption at 1740, 1715 and 1680 cm^{-1}. Compound **9** would thus appear to be a hydroxy derivative of **5**. This is borne out by the mass spectrum of **9** (Scheme 4). The molecular ion (*m/z* 572) loses two molecules of acetic acid (in the manner of cyathic acid **5**) to give a fragment at *m/z* 452. This ion gives rise to three further fragments, two at *m/z* 219 ($C_{14}H_{19}O_2$ (24), $C_{15}H_{23}O$ (16)) And one at *m/z* 234 ($C_{16}H_{26}O$). One ion at *m/z* 219 ($C_{14}H_{19}O_2$) is similar to that found for **5**,


-2 CH3CO2H
m/z 572 C34H52O7
m/z 452 C30H44O3
A
m/z 219 C14H19O2 (24)
m/z 189 C14H21 (14)
m/z 234 C16H26O (70)
m/z 219 C15H23O (16)

Scheme 4.

implying that the A and B rings of cyathic acid and **9** are the same. The absence of the *m/z* 218 fragment observed in the MS of cyathic acid and the presence of fragments at *m/z* 234 and *m/z* 219 ($C_{15}H_{23}O$) in the MS of **9** indicates that the locus of the additional hydroxyl group in **9** is the CDE rings.

The ^{1}H NMR spectrum of **9** shows many similarities with that of cyathic acid, i.e. 5 angular methyl groups, a vinylic methyl and two acetyl methyls. Besides the low field vinyl protons and acetoxyl carbon protons there is a hydroxyl carbinyl proton as a doublet (δ3.62) with a small coupling (J = 5 Hz) suggesting that it is an equatorial proton and that the hydroxyl group is thus axial or that it is located in the 4-membered ring.

The hydroxyl group of **9** was shown to be at C-22 by comparison of the ^{13}C NMR spectrum of pygameic acid **9** with that of cyathic acid **5**: One observes four carbon perturbations (two upfield, two downfield). The most informative changes are the upfield shifts at C-16 (−8.0 ppm) and C-18 (−5.5 ppm). These large upfield shifts can only be explained by the removal of a synclinal interaction between C-16 and C-22. In particular, the shift of C-16 removes the possibility that the hydroxyl group of pygmaeic acid is at C-21 (C-16 is δ to C-21). Thus pygmaeic acid is 22-α-hydroxycyathic acid and furthermore the shifts observed in the ^{13}C NMR spectrum of **9** agree well with predicted values (Fig. 1) as reported by Wenkert *et al.*[10] for the 17-hydroxysteroids.

Oxidation of **9** with Jones' reagent gave the keto acid **28**. (IR: 1740, 1680 cm^{-1}). The position of the ketone group in **28** was determined by examination of its ^{13}C NMR spectrum (Table 2). Within the cyathic acid series certain carbons appear as characteristic resonances, e.g. C-10, C-14, C-17 are singlets at ~43 ppm. In the ^{13}C NMR of **28** one of these singlets has shifted downfield 7.2 ppm. Since the carbon resonances for C-11, C-12 and C-8, C-15 are unchanged, C-10 and C-14 cannot have shifted. This implies that the carbonyl group of **28** is located adjacent to C-17. Of the various possibilities, a carbonyl at C-22 best fits the observed perturbations (Fig. 1).[10] This confirms pygamaeic acid **9** as 22-hydroxycyathic acid. Furthermore the stereochemistry of the hydroxyl group in **9** was established on the basis of the observed pyridine shift[11] in the ^{1}H NMR of pygmaeic acid **9**. Since C-30 (vinylic methyl) showed a shift of −0.20 ppm and C-28 (angular methyl) showed a small shift of −0.06 ppm we can conclude that the hydroxyl group and C-28 are antiperiplanar. The large shift observed for C-30 must be due to the spatial proximity of these groups.

Compound **9** forms a methyl ester **29** (CH_2N_2) which can be converted into a triacetate **30** (Ac_2O/py) or a mesylate **31** (MsCl/py). Reduction of compound **31** with $LiAlH_4$ gave a mixture of compounds, a minor component of which was identical (TLC, MS) with compound **11**, the diol derived from methyl cyathate.

Triterpenes **5–9**, isolated from two species of *Cyathus* represent a new series of oxidized lupenes. These compounds are the first reported lupenes which bear functionality at C-26.

EXPERIMENTAL

M.ps were measured on a Leitz microscope m.p. apparatus and are uncorrected. IR spectra were recorded as chloroform casts on a Nicolet 7199 FT spectrometer. Mass spectra were recorded on an AEI MS-50 high resolution mass spectrometer coupled to a DS-50 computer and are given as *m/z* (rel int). Determinations of composition of fragment ions are accurate within ±3.5 ppm. NMR spectra were recorded on a Bruker WH 400 spectrometer, a Bruker WH 200 spectrometer or on a Varian HA 100 spectrometer interfaced to a Digilab FT/NMR 3 data system.

Preparative TLC (PTLC) were performed on Si gel G 60 (E. Merck, Darmstadt) with a thickness of 0.5 mm, containing 1% electronic phosphor. The plates were visualized by UV light or by spraying with 1% vanillin in conc H_2SO_4. The adsorbed compounds were removed with CH_2Cl_2 containing 10% MeOH.

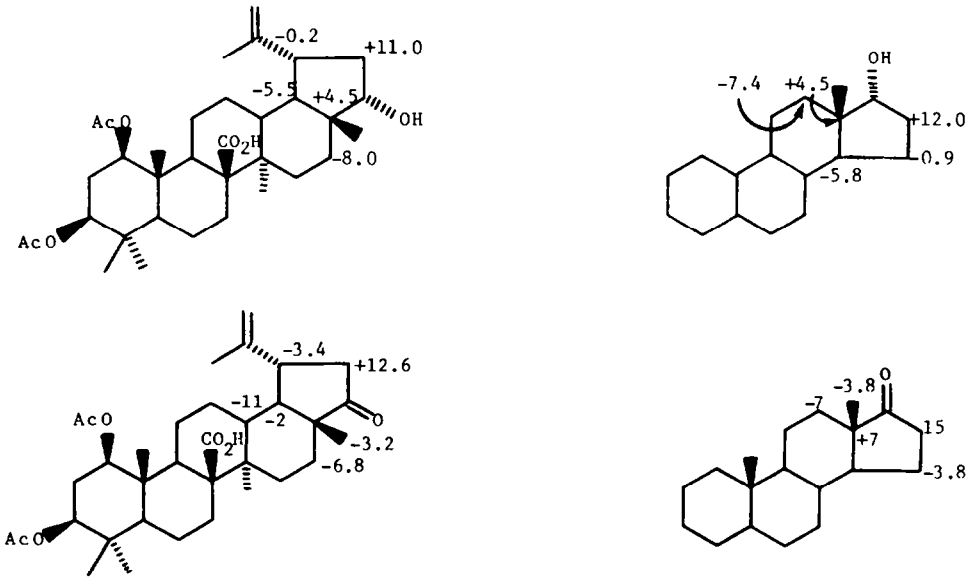


Fig. 1.

Extraction of Cyathus

Mycelium from agar slants of *Cyathus striatus* Willd. ex Pers. 68037 (ATCC 38356) or of *Cyathus pygmaeus* Lloyd 66133 (ATCC 38354) was transferred to a 250 ml Erlenmeyer flask containing 150 ml of sterilized Brodie's medium[12] having the following composition per litre: maltose, 5.0 g; dextrose, 2.0 g; yeast extract (Difco), 2.0 g; KH_2PO_4, 0.5 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.5 g; $MgSO_4$, 0.2 g; peptone, 0.2 g; asparagine, 0.2 g; $Fe_2(SO_4)_3$, trace. The flask was kept at 20–24° on a rotary shaker for 14 days. The contents of one such flask was used to inoculate 10 L of Brodie's medium which was then grown 15 days at 22° on a New Brunswick Scientific microfermentation apparatus with stirring (200 rev./min) and aeration (3 L/min.). To prevent foaming 1 mL of polypropylene glycol (av mol wt ~2000) was added.

The mycelium was separated from the broth by filtration and was extracted with acetone in a Soxhlet extractor. The acetone extract was partitioned three times between a small volume of water and ten volumes of EtOAc. The combined organic phases were evaporated to dryness giving 1.3 g (*C. striatus*) and 1.70 g (*C. pygmaeus*) per 10 L fermentation. The MeOH soluble part of the mycelial extracts from 3 × 10 L fermentations of *C. striatus* (2.30 g) was placed on a column of Sephadex LH20 (200 g, length 70 cm) in MeOH. Ten mL fractions were collected. The cyathic acid derivatives were found in fractions 16–22 together with other triterpenes and sesquiterpenes. Repeated separation of selected fractions by column chromatography over Si gel eluting with $CHCl_3$ or $CHCl_3$–Et_2O gave the pure compounds **5**, **6**, **7** and **8** as well as **2**. The MeOH insoluble part (1.60 g) could be fractionally crystallized to give glochidonal (**2**, 1.37 g) and cyathic acid (**5**, 0.23 g). The mycelial extract from *C. pygmaeus* (3 × 10 L fermentation) was treated similarly to give 3.30 g of MeOH soluble material which furnished compounds **5** and **9** as well as the lupenes **1**, **2**, **3** and **4**. The MeOH insoluble part contained almost pure glochidonol (**2**).

The broth was extracted 5 times with half its volume of EtOAc. The extracts were combined and evaporated to dryness. Yield per 3 × 10 L fermentation 5.4 g (*C. striatus*) and 3.54 g (*C. pygmaeus*) of which 0.45 g was insoluble in MeOH. These extracts were separated over Sephadex LH20 in MeOH and furnished upon purification as described above cyathic acid derivatives as well as lupenes.

C. striatus, when grown in still cultures in Brodie's medium for 120 days, furnished compounds **5**, **6** and **7** in low yields (1.46 g from 23 L), but the relative amount of **7** was increased.

Cyathic acid (**5**)

Yield: *C. striatus* fermentation 145 mg/10 L, still culture 16 mg/10 L; *C. pygmaeus* fermentation 39 mg/10 L, still culture 48 mg/10 L. M.p. 311–312 (subl 280°). Found: C, 72.39; H, 9.46. Calc for $C_{34}H_{52}O_6$: C, 72.35; H, 9.41%. IR: ν_{max} 3500–2700 (broad), 2960 (s), 1740 (s), 1685, 1380 and 1245 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ4.68 (s, 1H), 4.6 (m, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.68 (broad s, 3H), 1.02 (s, 6H), 0.85 (s, 6H), 0.73 (s, 3H). MS: $M^+ = 556$ ($C_{34}H_{52}O_6$, 36%), 496 (9), 436 ($C_{30}H_{44}O_2$, 30), 219 ($C_{14}H_{19}O_2$, 34), 218 ($C_{16}H_{26}$, 100), 203 (79), 189 (75), 175 (43), 161 (33), 149 (35), 147 (47), 135 (91). (UV (MeOH) no absorption above 210 nm). CD (*c*, 1.9 g/mL, MeOH): $[\theta]_{380}$ 0; $[\theta]_{342}$ −0.234; $[\theta]_{300}$ 0; $[\theta]_{250}$ 0.409; $[\theta]_{240}$ 0; $[\theta]_{224}$ −14.63. $[\alpha]_D$ (*c* 2.6 mg/mL, MeOH) 12.6°.

Methyl cyathate (**10**)

Treatment of **5** with excess CH_2N_2 in CH_2Cl_2 for 12 hr gave **10** in quantitative yield. Compound **10** was recrystallized from MeOH. M.p. 235°. IR: ν_{max} 2975 (s), 1740 (s), 1719 (s), 1250 and 1240 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ4.7–4.4 (m, 4H), 3.70 (s, 3H), 2.0 (s, 3H), 1.95 (s, 3H), 1.67 (broad s, 3H), 0.98 (s, 3H), 0.85 (s, 3H), 0.82 (s, 6H), 0.69 (s, 3H). MS: $M^+ = 570$ ($C_{35}H_{54}O_6$, 100), 510 (28), 450 ($C_{31}H_{46}O_2$, 22), 391 (18), 246 ($C_{16}H_{22}O_2$, 29), 233 ($C_{15}H_{21}O_2$, 44), 218 ($C_{16}H_{26}$, 73) 203 (49), 189 (48), 147 (30), 135 (48), 121 (52), 107 (55), 93 (53).

Methyl 1β,3β-dihydroxylup-20,29-en-26-oate (**11**)

To a solution of **10** (20 mg, 0.035 mmol) in dry Et_2O (10 mL) was added $LiAlH_4$ (20 mg, 0.53 mmol). The mixture was stirred 24 hr at room temp. Upon workup with aq. $NaHCO_3$ (20 mL) the Et_2O layer was removed and the aqueous phase was extracted twice with Et_2O. The extracts were dried (Na_2SO_4) and concentrated to give 15.7 mg (92%) of **11**, m.p. 131–133° ($CHCl_3$–hexane). IR: ν_{max} 3390, 2950, 1718 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ4.59 (s, 1H), 4.48 (s, 1H), 3.69 (s, 3H), 3.44 (dd, 1 H, J = 10 and 5 Hz), 3.21 (dd, 1H, J = 12 and 4.5 Hz), 1.68 (s, 3H), 1.00 (s, 3H), 0.92 (s, 3H), 0.71 (s, 6H), 0.68 (s, 3H). MS: $M^+ = 486$ ($C_{31}H_{50}O_4$, 100), 443 (26), 426 (33), 269 (27), 218 (60), 203 (73), 189 (73), 175 (41).

Methyl 1β,3β-dimethoxylup-20,29-en-26-oate (**12**)

Methyl 1β,3β-dihydroxylupenoate (**11**) (41 mg, 0.084 mmol) was dissolved in dry DME and excess MeI and NaH was added. The mixture was allowed to stir for 48 hr. The mixture was treated with MeOH, concentrated, dissolved in H_2O and extracted with Et_2O. Concentration of the Et_2O extract gave a mixture of two monomethylated and one dimethylated compounds. Separation by PTLC (Silica gel, benzene–Et_2O, 3:1) gave **12** (32 mg, 75%, R_f 0.7). IR: ν_{max} 2960, 1720, 1460, 1100, 1080 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz), δ4.70 (s, 1H), 4.60 (s, 1H), 3.68 (s, 3H), 3.35 (s, 3H), 3.23 (s, 3H), 2.73 (dd, 1H, J = 11 and 5 Hz), 2.59 (dd, 1H, J = 12 and 4 Hz), 1.70 (s, 3H), 1.00 (s, 3H), 0.91 (s, 3H), 0.72 (s, 3H), 0.70 (s, 6H). MS: $M^+ = 514$ ($C_{33}H_{54}O_4$, 100), 482 (45), 454 (12), 297 ($C_{17}H_{29}O_4$, 22), 233 (19), 218 ($C_{16}H_{26}$, 49), 203 (55), 189 (40).

1β,3β-Dihydroxylup-20,29-en-26-oic acid (**13**)

Reduction of **5** as described above gave **13** in 87% yield. Compound **13** was purified by sublimation, m.p. 307–309°. IR: ν_{max} 3400, 2960, 1685 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz), δ4.66 (s, 1H), 4.60 (s, 1H), 3.8–3.4 (m, 2H), 1.7 (s, 3H), 1.05 (s, 3H), 0.93 (s, 3H), 0.88 (s, 3H), 0.72 (s, 6H). MS: $M^+ = 472$ ($C_{30}H_{48}O_4$, 65), 457 (21), 454 (19), 429 (39), 219 (14), 218 (67), 203 (77), 189 (74), 175 (50), 135 (83), 136 (69), 121 (100).

1,3-Dioxolup-20,29-en-26-oic acid (**14**)

Dihydroxylupenoic acid **13** (2 mg, 0.004 mmol) was dissolved in acetone and treated with excess (1 drop) Jones' reagent. The solution was stirred for 5 min and excess reagent was removed by addition of isopropanol (4 drops). The reaction mixture was concentrated and the residue was dissolved in CH_2Cl_2 and washed with H_2O, dried ($MgSO_4$) and concentrated to give compound **14**, 1.8 mg (91%), m.p. 285–286° from MeOH. UV (MeOH) λ_{max}: 257 (ϵ = 10,000); (MeOH + NaOH (aq)) λ_{max} 288 (ϵ = 26,000). IR: 2960, 1720, 1698, 1676 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ4.67 (s, 1H), 4.58 (s, 1H), 3.38 (s, 2H), 1.70 (s, 3H), 1.15 (s, 3H), 1.11 (s, 3H), 1.01 (s, 3H), 0.74 (s, 3H). MS: $M^+ = 468$ ($C_{30}H_{44}O_4$, 100), 218 (32), 203 (56), 189 (51), 175 (22), 153 (63), 121 (49).

1,3-Diacetoxylupan-26-oic acid (**15**)

PtO_2 (2 mg, 0.009 mmol) was added to a soln of cyathic acid (**5**) (14 mg, 0.03 mmol) in MeOH (10 mL). The mixture was hydrogenated at room temp and atmospheric pressure under stirring for 12 hr. The Pt was removed by filtration and the solution concentrated to give compound **15** (11.4 mg, 84%), m.p. 306–308° (MeOH). IR: ν_{max} 3300–3050 (br), 2970 (s), 1738 (s), 1685 (s), 1268 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz), δ4.8–4.5, 2.01 (s, 3H), 1.98 (s, 3H), 1.01 (s, 6H), 0.85 (br. s, 12H), 0.70 (s, 3H). MS: $M^+ = 558$ ($C_{34}H_{54}O_6$, 8), 498 (10), 438 ($C_{30}H_{46}O_2$, 47), 395 ($C_{27}H_{39}O_2$, 13), 220 ($C_{16}H_{28}$, 10), 219 ($C_{14}H_{19}O_2$, 34), 218 ($C_{14}H_{18}O_2$, 11), 191 (43), 149 (36), 135 (100), 123 (61), 109 (41), 107 (47).

Decarboxylation of cyathic acid

Compound **5** (24.1 mg, 0.043 mmol) was heated at reflux in benzene (10 mL) under N_2 with $Pb(OAc)_4$ (44.4 mg, 0.101 mmol). The reaction mixture was poured into 5 mL of 1% aq. $FeSO_4$ and extracted with Et_2O. The Et_2O extract was washed with H_2O, aq. $NaHCO_3$, H_2O, dried Na_2SO_4 and concentrated to give compound **16** (21 mg, 95%). IR: 2950, 1740 (s), 1240 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ5.44 (m, 1H), 4.8 (dd, 1H, J = 11 and 4.5 Hz), 4.62 (m, 3H), 2.05 (s, 6H), 1.69 (s, 3H), 0.97 (s, 3H), 0.95 (s, 6H), 0.88 (s, 3H), 0.8 (s, 3H). CI: $M^+ = 510$ ($C_{33}H_{50}O_4$). MS: $M^+ - C_2H_4O_2$, 450 ($C_{31}H_{46}O_2$, 46), 390 (100), 375 (52), 321 (50).

Striatic acid (**6**)

Yield: *C. striatus* fermentation 365 mg/10 L, still culture 28 mg/10 L, m.p. 293–295.5° from MeOH. IR: ν_{max} 3400 (broad), 2960, 1730 (s), 1680 (s), 1455, 1370, 1240 (s). ^1H NMR ($CDCl_3$, 100 MHz), δ4.4 (m, 3H), 3.3 (dd, 1H, J = 11 and 4 Hz), 2.01 (s, 3H), 1.69 (s, 3H), 1.0 (s, 3H), 0.97 (s, 3H), 0.79 (s, 3H), 0.73 (s, 3H). MS: $M^+ = 514$ ($C_{32}H_{50}O_5$, 59%), 499 (13), 454 (26), 436 (12), 219 ($C_{14}H_{19}O_2$, 25), 218 ($C_{16}H_{26}$, 100), 203 (80), 189 (73), 175 (40). $[\alpha]_D$ (*c* 3.3 mg/mL, MeOH) −21.2°.

Acetylation of striatic acid

Treatment of compound **6** (6 mg, 0.012 mmol) with Ac_2O (1 mL) and pyridine (3 drops) gave upon workup cyathic acid (**5**) (5 mg, 75%), m.p. 307–310°, which compared satisfactorily on TLC, ^1H NMR, IR and MS with natural cyathic acid **5**.

Methyl striatate (**22**)

Treatment of striatic acid **6** (13 mg, 0.025 mmol) with CH_2N_2 in CH_2Cl_2 for 18 hr followed by concentration gave white crystals (13.5 mg, 100%), m.p. 206–209° from MeOH. IR: ν_{max} 3400 (br), 2960 (s), 1720 (s), 1240 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz), δ4.67 (s, 1H), 4.58 (s, 1H), 4.54 (dd, 1H, J = 11 and 5 Hz), 3.69 (s, 3H), 3.26 (dd, 1H, J = 12 and 4.5 Hz), 1.98 (s, 3H), 1.67 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.83 (s, 3H), 0.74 (s, 3H), 0.69 (s, 3H). MS: $M^+ = 528$ ($C_{33}H_{52}O_5$, 100), 468 (47), 233 (25), 218 (41), 203 (34), 189 (27).

Partial hydrolysis of methyl cyathate

Dry K_2CO_3 (50 mg, 0.36 mmol) was added to a solution of methyl cyathate (**10**) (20 mg, 0.035 mmol) in MeOH. The mixture was stirred rapidly for 30 min. and then left for 8 hr. The reaction mixture was filtered, concentrated, redissolved in H_2O, extracted with CH_2Cl_2, dried and concentrated to give compound **22** (16.7 mg, 90%), m.p. 208–210° from MeOH identical with an authentic sample of methyl striatate (**22**).

Epistriatic acid (**8**)

Yield: *C. striatus* fermentation 2 mg/10 L still culture 6 mg/10 L, m.p. 294–297° from MeOH. IR: ν_{max} 3500 (br), 2960, 1730 (s), 1710 (s), 1675 (s), 1240 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ4.90 (dd, 1H, J = 9.5 and 5.5 Hz), 4.63 (m, 2H), 3.51 (dd, 1H, J = 2.5 and 3.5 Hz), 1.98 (s, 3H), 1.67 (s, 3H), 1.04 (s, 3H), 1.01 (s, 3H), 0.93 (s, 3H), 0.84 (s, 3H), 0.72 (s, 3H). MS: $M^+ = 514$ ($C_{32}H_{50}O_5$, 57), 499 (17), 454 ($C_{30}H_{46}O_3$, 47), 439 (14), 436 (13), 219 ($C_{14}H_{19}O_2$, 35), 218 ($C_{16}H_{26}$, 100), 203 (87), 189 (79) 175 (42). $[\alpha]_D$ (*c* 1.1 mg/3 mL, MeOH) 11.1°.

Methyl epistriatate (**24**)

Treatment of compound **8** (2.1 mg, 0.004 mmol) with CH_2N_2 in CH_2Cl_2 for 15 hr, concentration and purification (PTLC, Si gel, benzene/ether, 5/1, 2 elutions) gave compound **24** R_f 0.37, 1.5 mg (70%). ^1H NMR ($CDCl_3$, 100 MHz): δ4.93 (dd, 1H, J = 9 and 5.5 Hz), 4.64 (m, 2H), 3.72 (s, 3H), 3.5 (dd, J = 2 and 3 Hz), 1.98 (s, 3H), 1.01 (s, 3H), 0.92 (s, 3H), 0.85 (s, 3H), 0.84 (s, 3H), 0.71 (s, 3H).

Oxidation of methyl epistriate (**24**)

Methyl epistriate **24** (2.4 mg, 0.0045 mmol) was dissolved in acetone (2 mL) and oxidized at 0°C for 20 min with a slight excess of Jones' reagent. MeOH (3 drops) was added, the mixture was diluted with 5 mL of H_2O, extracted with CH_2Cl_2, dried (Na_2SO_4) and concentrated to give 1.9 mg (80%) of methyl 1β-acetoxy-3-oxolup-20,29-en-26-oate (**25**). IR: ν_{max} 2960, 1718 (broad and strong), 1240 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ4.97 (dd, 1H, J = 8 and 2Hz), 4.65 (s, 1H), 4.57 (s, 1H), 3.73 (s, 3H), 3.08 (dd, 1H, J = 15 and 8 Hz), 2.02 (s, 3H), 1.68 (s, 3H), 1.06 (s, 3H), 1.02 (s, 6H), 0.70 (s, 6H). MS: $M^+ = 526$ ($C_{33}H_{50}O_5$, 31), 467 (21), 466 (36), 406 (10), 218 (18), 203 (37), 189 (32), 175 (20), 93 (100).

Oxidation of methyl striate

Oxidation of methyl striatate **22** as described above gives compound **25** identical in all respects with that obtained by oxidation of methyl epistriatate **24**.

Methyl 1β,3α-diacetoxylup-20,29-en-26-oate (**26**)

Methyl epistriate **24** (1.5 mg, 0.0029 mmol) was dissolved in Ac_2O (0.5 mL) and 1 drop of pyridine was added. The mixture was allowed to stand 15 hr and concentrated to give compound **26** (1.6 mg). Upon comparison, compound **26** was found to differ from methyl cyathate **10** by TLC and ^1H NMR. ^1H NMR ($CDCl_3$, 100 MHz): δ4.70 (m, 4H), 3.72 (s, 3H), 2.11 (s, 3H), 1.97 (s, 3H), 1.67 (s, 3H), 1.04 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.71 (s, 3H).

Cyathadonic acid (**7**)

Yield: *C. striatus* fermentation 11 mg/10 L) m.p. 255–257° (MeOH). UV: ν_{max} MeOH, 230 nm (ϵ6400). IR: ν_{max} 3300–2900 (broad), 2960, 1676 (s). ^1H NMR ($CDCl_3$, 100 MHz): δ7.19 (d, 1H, J = 10 Hz), 5.85 (d, 1H, J = 10 Hz), 4.69 (complex, 2H), 1.70 (s, 3H), 1.16 (s, 3H), 1.05 (s, 6H), 1.03 (s, 3H), 0.72 (s, 3H). MS: $M^+ = 452$ ($C_{30}H_{44}O_3$, 100), 437 (14), 218 (25), 203 (56), 189 (40).

Methyl cyathadonate (**27**)

Cyathadonic acid **7** (6 mg, 0.013 mmol) was methylated with CH_2N_2 in CH_2Cl_2 for 12 hr. Concentration gave methyl cyathadonate **27** (6.1 mg, 100%), m.p. 175–178° IR: ν_{max} 2940, 1716, 1671, 1455, 1220 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ7.17 (d, 1H, J = 10 Hz), 5.82 (d, 1H, J = 10 Hz), 4.64 (comp. 2H), 3.72 (s, 3H), 1.70 (s, 3H), 1.13 (s, 3H), 1.04 (s, 6H), 0.90 (s, 3H), 0.73 (s, 3H). MS: $M^+ = 466$ ($C_{31}H_{46}O_3$, 100), 451 (16), 423 (16), 406 (22), 218 (15), 203 (54), 189 (41).

Conversion of methyl 1β-acetoxy-3-oxolup-20,29-en-26-oate (**25**) *into methyl cyathadonate* (**27**)

Compound **25** (8 mg, 0.015 mmol) was treated with 20% aq. NaOH (2 drops) in MeOH (2 mL) for 15 min. The reaction mixture was acidified with 6 M HCl, concentrated, redissolved in CH_2Cl_2, washed with H_2O, dried (Na_2SO_4), and concentrated to give a compound (5 mg, 72%), m.p. 172–175°, identical in all respects with methyl cyathadonate (**27**).

Pygmaeic acid (**9**)

Yield: *C. pygaeus* fermentation 7 mg/10 L still cultures 8 mg/10 L. M.p. 270–272° (MeOH). IR: ν_{max} 3400 (br), 3300–2400 (br), 2960, 1740, 1715, 1680, 1370, 1240 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ4.6 (complex, 4H), 3.63 (d, 1H, J = 5 Hz), 2.01 (s, 3H),1.96 (s, 3H), 1.70 (s, 3H), 1.04 (s, 3H), 1.00 (s, 3H), 0.83 (s, 6H), 0.67 (s, 3H). MS: $M^+ = 572$ ($C_{34}H_{52}O_7$), 554 (11), 452 ($C_{30}H_{44}O_3$, 12), 434 (7), 234 ($C_{16}H_{26}O$, 70), 219 ($C_{14}H_{19}O_2$, 29, $C_{15}H_{23}O$, 16), 216 ($C_{16}H_{24}$, 33), 205 (16), 201 (22), 189 (14). $[\alpha]_D$ (*c* 2.5 mg/mL, MeOH), 10.8°.

1β,3β-Diacetoxy-22-oxolup-20,29-en-26-oic acid (**28**)

Oxidation of pygmaeic acid **9** (23 mg, impure, 0.02 mmol) with excess Jones' reagent (1 drop in acetone for 20 min at 0°) followed by addition of MeOH, H_2O and extraction with

CH_2Cl_2 gave 21 mg of a crude mixture. Separation on PTLC (silica gel, benzene–ether, 1:1) gave keto acid **28** (6 mg, 50%, R_f 0.55). IR: ν_{max} 3400–2400 (br), 2960, 1740 (s), 1680, 1245 (s) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ4.85 (m, 1H), 4.74 (m, 1H), 4.66 (dd, 1H, J = 11 and 5 Hz), 4.58 (dd, 1H, J = 12 and 4 Hz), 2.75 (dd, 1H, J = 10 and 5 Hz), 2.6 (m, 2H), 2.37 (d, 1H, J = 12 Hz), 2.04 (s, 3H), 2.0 (s, 3H), 1.70 (s, 3H), 1.02 (s, 3H), 1.01 (s, 3H), 0.89 (s, 3H), 0.84 (s, 6H). MS: M^+ = 570 ($C_{34}H_{50}O_7$, 16), 510 (11), 450 (69), 435 (15), 405 (17), 404 (17), 232 (32), 219 (100), 203 (21), 189 (33).

Methyl pygmaeate (**29**)

Compound **9** (2.4 mg, 0.004 mmol) was treated with excess CH_2N_2 in CH_2Cl_2 for 16 hr. The reaction mixture was evaporated to dryness to give 2.6 mg of material. ^1H NMR ($CDCl_3$, 100 MHz): δ4.66 (complex, 4 H), 3.71 (s, 3H), 3.6 (compl. 1H), 2.02 (s, 3H), 1.96 (s, 3H), 1.69 (s, 3H), 1.01 (s, 3H), 0.85 (s, 3H), 0.82) (s, 6H), 0.65 (s, 3H).

Methyl 1β,3β, 22α-triacetoxycyathate (**30**)

Acetylation of **29** (2.6 mg, 0.004 mmol) with Ac_2O and pyridine for 14 hr at room temp gave compound **30** in quantitative yield. ^1H NMR ($CDCl_3$, 400 MHz): δ4.76 (s, 1H), 4.70 (d, 1H, J = 5 Hz), 4.66 (dd, 1H, J = 10 and 4.5 Hz), 4.65 (s, 1H), 4.59 (dd, 1H, J = 12 and 4.5 Hz), 3.72 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.70 (s, 3H), 1.02 (s, 3H), 0.88 (s, 3H), 0.86 (s, 6H), 0.75 (s, 3H). MS: M^+ = ($C_{37}H_{56}O_8$, 22), 568 (100), 508 (24), 448 ($C_{31}H_{44}O_2$, 16), 233 ($C_{15}H_{21}O_2$, 30), 216 ($C_{16}H_{24}$, 78), 203 (14), 189 (15), 187 (35), 173 (19).

1β-Acetoxy-3β-hydroxylupene (**18**)

Sodium borohydride (15 mg, 0.4 mmol) was added to a solution of glochidonol acetate (**17**) (185 mg, 0.38 mmol) in dry THF (20 mL). The mixture was stirred at room temp for 24 hr. The THF was removed under reduced pressure and the residue dissolved in a mixture of sat. $NaHCO_3$ soln (20 mL) and CH_2Cl_2 (20 mL). The organic layer was drawn off and the aqueous layer extracted twice with CH_2Cl_2. The CH_2Cl_2 solution was dried (Na_2SO_4) and concentrated to give 1β-acetoxy-3β-hydroxylupene (**18**, 159 mg, 86%). This was recrystallized from MeOH to give white clusters, m.p. 215–217°. IR: ν_{max} 3450, 2970, 1720, 1250 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ4.5 (m, 3H), 3.3 (d of d, 1H, J = 14 and 5 Hz), 2.0 (s, 3H), 1.68 (b s, 3H, 1.03 (s, 3H), 1.00 (s, 3H), 0.96 (s, 3H), 0.92 (s, 3H), 0.78 (s, 6H). MS: M^+ = 484 ($C_{32}H_{52}O_3$, 7), 424 (37), 189 (21), 135 (22), 121 (33), 109 (37), 107 (33), 95 (40), 93 (31).

Lupanol (**20**)

Sodium borohydride (13 mg, 0.34 mmol) was added to a soln of lupanone (**19**) (140 mg, 0.33 mmol) in dry THF (50 mL). The mixture was stirred for 12 hr, then concentrated. The residue was redissolved in a mixture of H_2O (20 mL) and CH_2Cl_2 (20 mL). The organic layer was removed and the H_2O layer extracted twice with CH_2Cl_2. The CH_2Cl_2 soln was dried (Na_2SO_4) and concentrated to yield pure lupanol (**20**, 119 mg, 85%). IR: ν_{max} 3380, 2950 cm^{-1}. ^1H NMR ($CDCl_3$, 100 MHz): δ3.2 (dd, 1H, J = 10, 6 Hz, CHOH), 1.9–0.6 (m, 51 H). MS: M^+ = 428 ($C_{30}H_{52}O$, 70), 207 (58), 189 (43), 135 (47), 123 (56), 121 (48), 109 (51), 95 (92), 93 (61), 69 (100).

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