Tetrahedron Vol. 41, No. 6, pp. 1067 to 1072, 1985
Printed in Great Britain.

0040-4020/85 $3.00 + .00

NOVEL ACETOXYCAPNELLENES FROM THE ALCYONACEAN *CAPNELLA IMBRICATA*†

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(*Received in USA 7 October* 1983)

Abstract—The isolation of eight novel acetylated capnellenes (**2**, **4**, **6**, **9**, **10**, **16**, **21** and **22**) by acetone extraction of fresh colonies of *C. imbricata* is described. They are the major sesquiterpenes present in the living animal. The previously reported capnellene polyols, isolated by CH_2Cl_2 or hexane extraction of sun-dried colonies, are shown to be artifacts, produced by (a) substrate-specific hydrolase(s) present in the soft coral. The hydrolase containing fraction is also able to catalyze the transfer of acetyl groups from several alkyl acetates to the capnellene polyols.

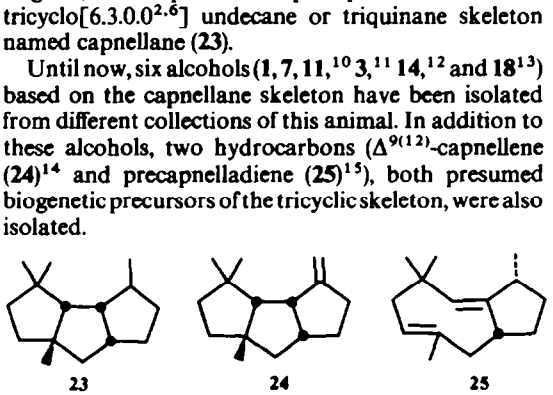
Soft corals of the order Alcyonacea (Anthozoa, Octocorallia) are abundant in the Indo–Pacific, where they can even be the major contributors to the biomass.[1] This chemical content has been systematically investigated during the 1970s[2] and about 70 sesquiterpenes and 200 diterpenes have so far been isolated from alcyonaceans, thus demonstrating that this group of marine animals, together with sponges,[3] constitutes a rich source of novel terpenoids. Like the related gorgonians, alcyonaceans mainly produce sesquiterpenes and cembrane-derived diterpenes.[2,4]

A number of these terpenoids exhibit interesting biological activities,[2] e.g. ichthyotoxicity, cytotoxicity, feeding repellency, thus suggesting that at least some of them possess a protective function for the colonies[2,5] and hence an important role in interspecific competition.[6]

Although much is known concerning the structures, distribution and biological activities of alcyonacean terpenoids, their biosynthetic origin still remains obscure. It is well known[7] that most Alcyonacea live in symbiotic relationship with intracellular algae, the zooxanthellae. These algae play a major role in the biology and in the energy balance of the colonies.[8] The terpenoids, then, might be produced by the symbiotic algae alone, by the animal, or by the association of both partners. To our knowledge, no biosynthetic work on alcyonacean terpenoids has been published yet. Incorporation experiments have been performed[9] on the sea-fan *Pseudoplexaura porosa*, a member of the Gorgonacea that are closely related to the Alcyonacea. In this case, the biosynthesis of crassin acetate (a cembrane diterpene) is accomplished solely by *P. porosa* zooxanthellae.[9] In surprising contrast, only the microsomal fraction derived from gorgonian tissues seems to be able to catalyze the formation of certain of the sesquiterpenes found in this animal.[9] Thus it appears that no simple scheme can be proposed yet to explain the biosynthetic origin of the octocoral terpenoids.

The terpenoid content of many species of Alcyonacea shows considerable variation.[1,2] The interpretation of these variations is not yet possible; individual, geographical and seasonal factors might be implicated. It is interesting to note that these variations generally arise in the functional groups and not in the skeleton. This has been well documented in the case of *Capnella imbricata* (Nephtheidea), a widely distributed member of the Alcyonacea whose study afforded an array of original, non-isoprenoid sesquiterpenes based on the tricyclo[6.3.0.$0^{2,6}$] undecane or triquinane skeleton named capnellane (**23**).

Until now, six alcohols (**1**, **7**, **11**,[10] **3**,[11] **14**,[12] and **18**[13]) based on the capnellane skeleton have been isolated from different collections of this animal. In addition to these alcohols, two hydrocarbons ($\Delta^{9(12)}$-capnellene (**24**)[14] and precapnelladiene (**25**)[15]), both presumed biogenetic precursors of the tricyclic skeleton, were also isolated.



23 **24** **25**

The peculiar structure of the capnellanes, like that of hirsutane,[16] constitutes a methodology test for synthetic chemists. Thus it is not surprising that, during the last two years, no less than six different approaches have been reported[17–22] for the synthesis of the capnellane skeleton.

The isolation procedure leading to the capnellane alcohols always involved maceration of sun-dried animal colonies in hexane or dichloromethane, followed by exhaustive extraction with the same solvent.[10] We recently observed that when ethyl acetate is used, the major sesquiterpenes are acetylated derivatives and not the free alcohols previously described.[10,13] This unexpected observation called for a careful reinvestigation of the secondary metabolites of *C. imbricata*. We now report on the structure determination of several novel acetoxycapnellenes and on evidence that most of the polyols and the acetyl derivatives isolated from sun-dried specimens are artifacts.

In a previous paper, we reported that the composition in sesquiterpenes of the dichloromethane or hexane extract of sun-dried *Capnella imbricata* varies with the place of collection.[10] Nevertheless, whatever the geographic origin, only polyhydroxylated sesquiterpenes were isolated. A recent and detailed

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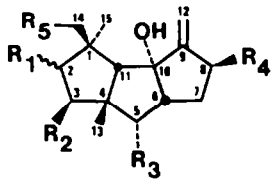
Table 1. List of oxygenated capnellenes



Compound		R_1	R_2	R_3	R_4	R_5
Diol	1	H	H	H	OH	H
	2	H	H	H	OAc	H
Triol	3	H	OH	H	OH	H
	4	H	OAc	H	OH	H
	5	H	OH	H	OAc	H
	6	H	OAc	H	OAc	H
Triol	7	H	H	OH	OH	H
	8	H	H	OH	OAc	H
	9	H	H	OAc	OH	H
	10	H	H	OAc	OAc	H
Triol	11	ξ-OH	H	H	OH	H
	12	ξ-OAc	H	H	OH	H
	13	ξ-OAc	H	H	OAc	H
Tetrol	14	H	OH	H	OH	OH
	15	H	OH	H	OAc	OH
	16	H	OAc	H	OH	OAc
	17	H	OAc	H	OAc	OAc
Tetrol	18	β-OH	H	OH	OH	H
	19	β-OAc	H	OH	OH	H
	20	β-OH	H	OH	OAc	H
	21	β-OAc	H	OAc	OH	H
	22	β-OAc	H	OAc	OAc	H

reexamination of sun-dried colonies of *C. imbricata* living around Laing Island (Papua, New Guinea), showed that individual colonies may be divided into two chemically distinct varieties. The dichloromethane extract of variety A contains the alcohols **1**, **7** and **18** together with small amounts of the triol **11**, whereas that of variety B contains as major compounds the alcohols **1**, **3** and **7** together with small amounts of the tetrols **14** and **18**. Both types of colonies have exactly the same colour, size or morphology and can only be distinguished from each other on the basis of their polyol content. TLC analysis of sun-dried colonies collected at Laing Island monthly during one year showed that there is no significant seasonal variation within a chemical variety.

When similar extractions with ethyl acetate as solvent were carried out on sun-dried individuals of each variety, the sesquiterpene fractions thus obtained proved much more complex. Indeed, together with the expected polyols a total of seven monoacetylated sesquiterpenes were detected. Six of them, namely **2**, **8**, **12**, **15**, **19** and **20** could be isolated in pure state by repetitive silica gel column chromatographies, whereas compound **5** was only detected by TLC and identified by comparison of its R_f value with that of a synthetic sample. Compound **2** was shown to be identical with the monoacetate previously obtained by acetylation of **1**.[10] The structures of the novel monoacetylated compounds (**8**, **12**, **15**, **19** and **20**) were established on the basis of their ^{1}H-NMR and mass spectra, as well as by acetylation into already known di- or triacetates. Compounds **8**, **12** and **15** are converted into **10**, **13** and **17**, respectively, whereas both **19** and **20** afford **22**. The distribution of these monoacetyl derivatives in the two chemical varieties of *Capnella imbricata* is reported in Table 3. It is worth noting that within each variety there exists a close structural relationship between their content in polyols (from dichloromethane extraction) and in monoacetates (from ethyl acetate extraction). Indeed, for each variety the monoacetylated polyols are those derived from the characteristic polyols of the variety by acetylation of the hydroxyl function at either C-8 or C-2.

A different situation prevails when fresh colonies of *C. imbricata* are extracted with acetone immediately after collection. In this case, the extract consists of a mixture of mono-, di- and triacetates (**2**, **9**, **10**, **21** and **22** for variety A and **2**, **4**, **6**, **9**, **10**, **16**, **21** and **22** for B), accompanied by small amounts of some of the corresponding polyols. These acetates (except **2**) are different from those obtained by extraction of sun-dried specimens with ethyl acetate. Their structures were deduced as previously from their ^{1}H-NMR and mass spectra and by acetylation into known derivatives. Their distribution in the two chemical varieties is also reported in Table 3. For each variety there is a close structural relationship between the content in polyols and acetates. Characteristically, a C-8 deacetyl derivative is associated to each peracetate.†

To explain these results, we must admit that the major compounds present in the living animal are the

† We call peracetate a capnellane derivative of which the primary and all the secondary hydroxyl groups are acetylated.

Table 2. NMR Data of the new acetoxycapnellenes (100 MHz, δ, J in Hz)

Compound	CH_3—C	CH_3—CO	CH_2=C	OH C_8—H	OAc C_8—H	OH C_x—H	OAc C_x—H
4	0.98–1.24–1.32	1.95	5.36	4.8m	—	—	C_3: 5.1 dd (J = 8, 12.5)
8	1.20–1.20–1.25	2.05	5.27	—	5.68m	C_5: 3.5 d (J = 5)	
9	1.12–1.12–1.21	2.03	5.38	4.8m	—	—	C_3: 4.6 d (J = 6.5)
12	1.19–1.31–1.37	2.09	5.42	4.7m	—	—	C_2: 4.8 dd (J = 6, 6)
15	0.98–1.32	2.05	5.39	—	5.83m	C_3: 4.17 dd (J = 6, 10.5) C_{14}: 3.52 q (J = 9)	
16	0.88–1.28	1.94–2.03	5.18	4.68m	—	—	C_3: 5.0 dd (J = 9, 9) C_{14}: 3.96 ABsyst (J = 10.5)
19	1.10–1.27–1.29	2.05	5.36	4.74m	—	C_5: 3.6 d (J = 3, 5)	C_2: 5.0 tr (J = 6)
20	1.10–1.28–1.30	2.08	5.37	—	5.73m	C_2: 4.03 tr (J = 6) C_5: 3.58 d (J = 3.5)	
21	1.12–1.22–1.25	2.03–2.06	5.43	4.8m	—	—	C_2: 4.85 dd (J = 6, 6) C_3: 4.66 d (J = 5)

peracetates **2**, **6**, **10**, **13**, **17** and **22** that are hydrolyzed into the corresponding polyols during sun-drying. Consequently, when sun-dried colonies are extracted later with dichloromethane or hexane, only the free alcohols are isolated. When fresh animals are treated with acetone, no or only partial hydrolysis occurs, leading to a mixture of peracetates and partly hydrolyzed peracetates. The fact that hydrolysis takes place mainly at C-8 indicates easier accessibility. This is to compare with the fact that *in vitro* smooth hydrolysis ($KHCO_3$–MeOH) of the peracetates also starts at the C-8 position, e.g. hydrolysis of **10** gives **9**; of **6** gives **4**; and of **22** gives **21**.

In addition we have to postulate that in the animal there exists a factor able to catalyze the transfer of the acetyl group of ethyl acetate to the hydroxyl function(s) of the capnellane alcohols. This transesterification reaction takes place mainly at the more accessible sites, C-8 and C-2.

The following experiments were conducted to examine the acetylating capability of *C. imbricata* and to define more accurately the active factor. First, we checked that the polyols **3**, **7** and **18** when dissolved in ethyl acetate and left at room temperature for 48 hr remain unchanged. These solutions were then stirred, in the same conditions, in the presence of sun-dried *C. imbricata* powder, previously extracted three times with ether to eliminate most of the sesquiterpene alcohols. Under these conditions, a transesterification took place, leading to the formation of the monoacetates **19** and **20** (20%), **8** (40%) and **5** (50%) starting from **18**, **7** and **3**, respectively. This proves the existence in the colonies of a factor able to acetylate the capnellane alcohols in the presence of added ethyl acetate. This acyl group transfer is characteristic of many esterases,[23] which normally catalyze the hydrolysis of carboxylic esters. Evidence that we are indeed dealing with an esterase in *C. imbricata* is as follows: sun-dried specimens of the animal were successively extracted with dichloromethane, methanol and water. TLC of the mixtures obtained by adding these extracts separately to an ethyl acetate solution of the triol **3**, revealed that only the aqueous extract is active. After lyophilization of the latter and fractionation of the resulting powder on a Sephadex G-25 column, we found that the transesterification activity was restricted to a fraction eluting shortly after the void volume of the column. As judged by Sephadex G-15 and G-25 filtration, the molecules constituting the active fraction must have a molecular weight between 1500 and 5000 daltons. This fraction is ninhydrin-positive and exhibits amide bands in the infrared and looses its activity after boiling for 1 hr, thus suggesting that the active principle is a polypeptide.

Furthermore, we checked the specificity of this esterase. Esterification experiments with other acetic acid esters, such as methyl acetate, butyl acetate or amyl acetate were attempted. To this end, solutions of triol **3** in each of these solvents were treated with a fraction of the lyophilized aqueous extract. TLC analysis revealed that formation of **5**, the C-8 monoacetate of **3**, takes place in all cases. In contrast, although esterification is observed when formic acid esters are used, no esterification takes place when the solvent is a propionic or butyric acid ester, or when the capnellane alcohols are replaced by another substrate. For example, no reaction occurred with menthol, 2-hydroxynephthenol,[24] 4-hydroxyclavulara-1(15),17-

Table 3. Distribution of the capnellenes into the different extracts of *C. imbricata*

	CH_2Cl_2 or hexane extract of sun-dried colonies		EtOAc extract of sun-dried colonies		Acetone extract of fresh colonies	
	Var A	Var B	Var A	Var B	Var A	Var B
1	***	***	**	**	**	*
2	—	—	**	**	**	*
3	—	***	—	**	—	—
4	—	—	—	—	—	*
5	—	—	—	*	—	—
6	—	—	—	—	—	*
7	***	***	**	**	*	*
8	—	—	**	**	—	—
9	—	—	—	—	*	*
10	—	—	—	—	**	*
11	*	—	*	—	*	—
12	—	—	*	—	—	—
14	—	*	—	*	—	—
15	—	—	—	*	—	—
16	—	—	—	—	—	*
18	**	*	*	*	—	—
19	—	—	*	*	—	—
20	—	—	*	*	—	—
21	—	—	—	—	*	*
22	—	—	—	—	**	*

diene[25] or cholesterol, even with prolonged reaction times.

It thus appears that the *C. imbricata* esterase has a high substrate specificity. Although hydrolases, and particularly esterases have usually been reported to exhibit broad substrate specificities,[23] recent investigations demonstrated that some animal and microorganism hydrolases possess exceptionally high specificities.[26]

Finally, we have shown that the fraction catalyzing the acetylation reactions also catalyzes the hydrolysis of the acetoxy groups of the capnellane, thus explaining the isolation of polyols on dichloromethane extraction of sun-dried animals. Indeed, when an ethereal solution of the peracetates **10**, **22** or **6** is stirred at room temperature in the presence of the active fraction, TLC analysis shows the progressive hydrolysis of the acetoxy group at C-8 leading to compounds **9**, **21** and **4**, respectively. The transformation is virtually complete after 48 hr. We do not know if both the hydrolysis and the transesterification are catalyzed by the same enzyme, as is the case for menthyl acetate esterase for example.[27]

Isolated *C. imbricata* zooxanthellae show esterase activity, suggesting that the enzyme may be located in the algae. This location may be the reason of the preservation of the activity in 3–4 y old dried specimens. Indeed, cell walls of the zooxanthellae are highly resistant to disruption and allow the algae to maintain their integrity even after desiccation and long storage.

As for the possible protective role of the capnellane sesquiterpenes against fish predation we have checked that the polyols and the corresponding acetates have comparable feeding deterrent activities against *Lebistes reticulatus*. Thus the fact that these sesquiterpenes are stored in the living animal mainly in the form of their acetates has no obvious ecological significance.

EXPERIMENTAL

Extraction procedures

(a) *From sun-dried animals.* The sun-dried soft corals, collected at Laing Island (Papua, New Guinea) were broken into small pieces and blended with hexane. The solid material was filtered and after overnight maceration was extracted with hexane. This treatment was repeated using either CH_2Cl_2 or EtOAc as solvent. The combined solutions were evaporated under reduced pressure, yielding dark brown oily residues.

(b) *From fresh animals.* Living colonies were cut into small pieces immediately after collection and covered with acetone. After filtration the solid material was rapidly extracted twice with acetone. The combined solutions were evaporated, yielding a dark green oily residue.

Isolation of the capnellenes and chromatographic analysis

The extracts were fractionated by flash chromatography using mixtures of hexane–acetone, chloroform–methanol or benzene–EtOAc as eluents. The fractions were analyzed by TLC using hexane–acetone (7:3), $CHCl_3$–MeOH (9:1) or benzene–EtOAc (8:2) as eluents, the capnellenes appearing as purple, pink or mauve spots upon visualization with ceric sulphate.

GC analyses were effected at 190° on a Hewlett-Packard HP402 gas chromatograph equipped with 3% OV-1 or OV-3 columns. The relative retention times (RRT) are calculated using **3** as internal standard (RT = 1).

Spectral analyses

The NMR spectra were recorded on a JEOL-100 spectrometer ($CDCl_3$ with TMS as internal standard). Chemical shifts (in δ values) are reported in Table 2.

Mass spectra were obtained with a VG-Organic 70/70 double focusing spectrometer. All peaks of relative intensity greater than 15% of the base peak are reported, except in the region of the high masses (over 200). Fragmentations associated with metastable transitions are noted by an asterisk *.

Characterization of the compounds

The following capnellenes are already described: 3;[11] 1, 2, 3, 6, 7, 10, 11, 13;[10] 14, 17;[12] 18, 22.[13]

3β-*Acetoxy*-$\Delta^{9(12)}$-*capnellene*-8β,10α-*diol* **4**: GC: RRT: 1.45. MS: $M^{+\cdot}$ 294 (0%), 234 (30, M − HOAc), 216 (40, 234 − H_2O), 201 (15, 216 − CH_3), 149 (35), 147 (22), 137 (68), 125 (46), 123 (62), 122 (50), 121 (48), 112 (30), 109 (100), 108 (40), 107 (66), 95 (40), 93 (43), 85 (33), 81 (36), 69 (36), 57 (35), 55 (40), 43 (60), 41 (38).

8β-*Acetoxy*-$\Delta^{9(12)}$-*capnellene*-5α,10α-*diol* **8**: GC: RRT: 1.26. MS: $M^{+\cdot}$ 294 (0%), 234 (20, M − HOAc), 216 (34, 234 − H_2O), 201 (16, 216 − CH_3), 183 (43, 201 − H_2O), 147 (38), 140 (54), 139 (50), 125 (74), 124 (86), 123 (84), 122 (33), 121 (36), 111 (30), 109 (70), 107 (53), 96 (52), 95 (80), 94 (70), 69 (54), 55 (50), 43 (100), 41 (50).

5α-*Acetoxy*-$\Delta^{9(12)}$-*capnellene*-8β,10α-*diol* **9**: m.p. 94–96° (hexane). GC: RRT: 1.10. MS: $M^{+\cdot}$ 294 (0%), 276 (1, M − H_2O), 252 (1, M − CH_2CO), 234 (50, M − HOAc), 219 (10, 234 − CH_3*), 216 (37, 234 − H_2O*), 201 (12, 216 − CH_3* and 219 − H_2O*), 182 (23), 165 (16), 147 (23), 140 (68), 125 (40), 123 (79), 122 (38), 121 (28), 112 (22), 109 (71), 107 (35), 95 (37), 69 (28), 55 (38), 43 (100), 41 (45).

2-*Acetoxy*-$\Delta^{9(12)}$-*capnellene*-8β,10α-*diol* **12**: GC: RT: 1.31. MS: $M^{+\cdot}$ 294 (0%), 234 (16, M − HOAc), 219 (13, 234 − CH_3*), 216 (41, 234 − H_2O*), 201 (12, 216 − CH_3* and 219 − H_2O*), 123 (77), 122 (56), 121 (27), 112 (53), 109 (100), 108 (55), 107 (81), 95 (22), 55 (22), 43 (75), 41 (31).

8β-*Acetoxy*-$\Delta^{9(12)}$-*capnellene*-3β,10α,14-*triol* **15**: GC: RRT: 3.80. MS: $M^{+\cdot}$ 310 (0%), 250 (2, M − HOAc), 232 (2, 250 − H_2O), 219 (1), 214 (1), 202 (4), 201 (5), 167 (15), 149 (15), 109 (40), 108 (70), 107 (84), 95 (56), 94 (84), 55 (42), 43 (100).

3β,14-*Diacetoxy*-$\Delta^{9(12)}$-*capnellene*-8β,10α-*diol* **16**: GC: RRT: 5.00. MS: $M^{+\cdot}$ 352 (less than 1%), 334 (1, M − H_2O), 292 (5, M − HOAc), 282 (6), 274 (8, 292 − H_2O*), 232 (8, 292 − HOAc* and 274 − CH_2CO*), 219 (4), 217 (3, 232 − H_2O*), 214 (10, 274 − HOAc* and 232 − H_2O*), 201 (8, 219 − H_2O*), 199 (5, 214 − CH_3*), 121 (50), 120 (42), 112 (35), 108 (56), 107 (100).

2β-*Acetoxy*-$\Delta^{9(12)}$-*capnellene*-5α,8β,10α-*triol* **19**: GC: RRT: 3.26. MS: $M^{+\cdot}$ 310 (0%), 250 (1, M − HOAc), 232 (3, 250 − H_2O), 214 (3), 199 (3), 141 (20), 138 (33), 123 (20), 121 (26), 109 (100), 95 (35), 43 (53).

8β-*Acetoxy*-$\Delta^{9(12)}$-*capnellene*-2β,5α,10α-*triol* **20**: GC: RRT: 2.80. MS: $M^{+\cdot}$ 310 (0%), 250 (6, M − HOAc), 232 (8, 250 − H_2O), 214 (12), 199 (6), 183 (15), 139 (18), 138 (19), 125 (20), 123 (22), 109 (30), 95 (27), 94 (32), 75 (32), 43 (100).

2β,5α-*Diacetoxy*-$\Delta^{9(12)}$-*capnellene*-8β,10α-*diol* **21**: m.p. 65–66° (hexane–ether). GC: RRT: 3.43. MS: $M^{+\cdot}$ 352 (0%), 334 (1, M − H_2O), 310 (1, M − CH_2CO), 292 (8, 352 − HOAc), 274 (8, 292 − H_2O*), 250 (2, 292 − CH_2CO*), 232 (6, 292 − HOAc*), 223 (8), 217 (17, 232 − CH_3*), 214 (22, 274 − HOAc* and 232 − H_2O*), 199 (13, 214 − CH_3*), 181 (27), 179 (18), 139 (21), 138 (50), 122 (18), 121 (100), 120 (40), 112 (35), 109 (78), 108 (28), 107 (29), 95 (21), 55 (19), 43 (95), 41 (18).

Acetylation and hydrolysis reactions

Acetylation. The compound (10 mg) was left overnight in 2 ml of Ac_2O–pyridine 1:3 mixture at room temp. After addition of water, the mixture was extracted with CH_2Cl_2 and the compounds purified by silica gel column chromatography.

Base hydrolysis. The acetate (10 mg) was dissolved in 20 ml of a saturated $KHCO_3$/MeOH soln and left for several days at room temp. After addition of water, the mixture was extracted with CH_2Cl_2 and the reaction products purified by silica gel column chromatography.

Preparation of the aqueous extract

(a) Several dried colonies of *C. imbricata* were ground in water. The suspension was filtered on Celite or centrifuged, yielding a clear solution.

(b) The aqueous phase obtained in (a) was extracted three times with CH_2Cl_2 or Et_2O to remove the bulk of sesquiterpene alcohols. However, polar compounds such as 7 were still present in the aqueous extract.

(c) The aqueous phase (10 ml) from (b) was lyophilized, redissolved in 2 ml of 0.02 M NH_4OAc buffer and applied to a Sephadex G-25 column that was equilibrated and developed (30 ml/hr, 3 ml fractions) with the same buffer. The transacetylase activity was found in the fractions eluted shortly after the void volume (peak maximum: 12 ml after the void volume). These fractions (free of capnellanes) were pooled and lyophilized, yielding 30 mg of a powder that was subsequently used in transacetylation assays. The transacetylase was shown to be totally excluded from Sephadex G-15 gel. The activity was lost after boiling for 1 hr or by treatment with methanol.

Acetylation reactions catalyzed by the aqueous extract

(a) A mixture of equal volumes (e.g. 2 ml) of the aqueous extract prepared in (a) or (b) above and of a 0.25% EtOAc soln of 3 was stirred at room temp for 48 hr. Analysis of the EtOAc phase shows the presence of 5 and 6, together with other acetylated derivatives (e.g. 8) which were not present in the aqueous extract.

(b) The 30 mg of powder obtained in (c) above was dissolved in 10 ml of water. Acetylation reactions were performed as described hereabove, with 2 ml of this solution. Only compounds 5 and 6 were formed in this procedure.

Feeding repellency tests

All tests were performed on the fish *Lebistes reticulatus* previously deprived of food for one day. Blanks were prepared by compressing 100 mg of Phillips "Superfood" fish food exactly as for preparing KBr pellets for IR spectroscopy. Test pellets were prepared by adding respectively 5% of triol 3, 5% of the corresponding diacetate 5, 1% of triol 7 or 1% of its diacetate 10.

The tests were performed in a 1 l beaker. Three pellets (one blank and two "actives") were separately disposed into small Petri covers and presented to five fish. During the next 20 min the number of times the fish swallowed or regurgitated the food was recorded. The experiment was repeated. A typical run gave the following results:

	Blank	3	5	Blank	7	10
Food swallowed	28	6	5	36	7	8
Food regurgitated	1	5	6	4	6	4

Acknowledgement—M.K. wishes to thank the "Fonds National de la Recherche Scientifique" for financial assistance towards travel.

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