New Propionate-Derived Metabolites from *Aglaja depicta* and from Its Prey *Bulla striata* (Opisthobranch Molluscs)

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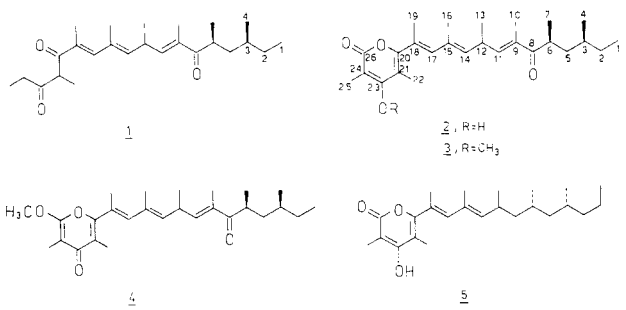
Received October 2, 1986

Two new polypropionate metabolites aglajne-2 (**14**) and -3 (**2**) and the previously known aglajne-1 (**1**) have been isolated from the opisthobranch mollusc *Bulla striata*, a prey of the opistobranch *Aglaja depicta* in which these compounds were first detected. This finding establishes the dietary origin of these metabolites in *A. depicta*. The structures were determined by extensive use of ^1H and ^{13}C NMR techniques on the compounds and on their derivatives and by chemical proofs. Two additional compounds (**12** and **13**), isolated as racemic diastereoisomers, are artifacts originating from aglajne-2 (**14**) during the extraction and purification procedures. The relative and absolute stereochemistry at C-3 and C-6 of aglajne-1 (**1**) and -3 (**2**) has been also determined.

Recent research has led to the isolation of several secondary metabolites from opisthobranch gastropods; it is believed that some of them play important ecological roles.[2] These compounds are usually of dietary origin, although several cases of de novo biosynthetic ability have been documented[3].

We have recently reported[4] the structure of aglajne-1 (**1**, without stereochemical implications), the major metabolite extracted from *Aglaja depicta* (Opisthobranchia, Bullomorpha, Philinacea). During this work the presence of other related metabolites was evidenced, but their instability prevented isolation and structural investigation.

A. depicta is a carnivorous mollusc. A macroscopic analysis of the digestive gland contents[5] revealed the presence of shells of other opisthobranch molluscs, *Bulla striata* (Bullomorpha, Bullacea) and *Haminea hydatis* (Bullomorpha, Atyidae). We therefore decided to investigate the metabolites of these two molluscs for comparison with those of *A. depicta*. We have found that the overall pattern of polypropionate metabolites contained in *B. striata* is the same as in *A. depicta*, hence establishing the dietary origin of the polypropionates extracted from the latter.[6] We have isolated from *B. striata* aglajne-1 (**1**),



extracted earlier from *A. depicta*, and two new propionate derived metabolites previously evidenced in the extracts of *A. depicta*, named[7] aglajne-2 and -3 in order of increasing polarity, whose structures are described in this paper.

Two additional compounds (**12** and **13**) were also isolated and shown to be artifacts originating from aglajne-2 (**14**) during the extraction and isolation procedures.

Aglajne-3 (**2**) the most polar component of the polypropionate mixture, $[\alpha]^{20}_D$ +105°, did not show a molecular ion in the mass spectrum. The ultraviolet absorption at longer wavelength (308 nm; ϵ 12 400) matched the value reported for diemensin-A (**5**), a metabolite previously isolated from the marine pulmonate *Siphonaria diemenensis*.[8] The ^1H NMR spectrum of **2** indicated analogy with both **1** and **5**. It contained five methyl groups in the δ 1.82–2.02 range, consistent with the presence of three vinyl methyls and two methyl groups on the pyrone ring, three olefinic protons at δ 6.43, 6.01, and 5.33, and two multiplets at δ 3.54 and 3.33 which were assigned to H-12 and H-6, respectively. The remaining methyl signals at δ 1.15, 1.02, 0.82, and 0.81 were interpreted as indicative for the presence in the molecule of a partial structure having similar features to the C-1–C-11 portion of aglajne-1 (**1**).

Since **2** was rather unstable, it was methylated with ethereal diazomethane to a mixture of two isomeric methyl ethers **3** and **4**, which were separated by SiO_2 flash chromatography. The molecular formula of both **3** and **4** was determined to be $C_{27}H_{40}O_4$ by HRMS.

The UV and IR data (Experimental Section), as well as the comparison of the ^{13}C NMR data (Table I) with those of similar compounds,[9] allowed us to assign the 4-methoxy-3,5-dimethyl-2*H*-pyran-2-one structure to the less polar pyrone **3** and the 2-methoxy-3,5-dimethyl-4*H*-pyran-4-one structure to the isomeric pyrone **4**. The chemical shift values of the methyl groups on the pyrone ring in the ^1H NMR spectra of the two compounds (Table I) further confirmed the structural assignments of the pyrone moieties, using similar arguments as for the methyl ether derivatives of **5**.[8]

Extensive use of spin decoupling and 2D COSY spectra of **3** and **4** allowed a complete assignment of all proton resonances as reported in Table I. A key observation was the irradiation of the C-13 methyl doublet which transformed the multiplet due to the C-12 proton into a triplet (J = 8.8 Hz) coupled with both the olefinic protons on C-11 and C-14, thus linking the conjugated diene to the enone system. An intense ion at m/z 233 ($C_{14}H_{17}O_3$ by HRMS) in the mass spectrum of both **3** and **4**, corresponding to the cleavage of the C_{12}–C_{14} bond, corroborates the conjugation of the diene to the pyrone moiety.

The comparison of the ^1H and ^{13}C NMR spectra of **3** and **4** (Table I) with those of aglajne-1 (**1**) confirmed the presence in these molecules, and hence in **2**, of the same

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(2) Faulkner, D. J.; Ghiselin, M. T. *Mar. Ecol. Prog. Ser.* **1983**, *13*, 295.
(3) Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G.; Villani, G. *Science (Washington, D.C.)* **1983**, *219*, 1237. Cimino, G.; De Rosa, S.; De Stefano, S.; Morrone, R.; Sodano, G. *Tetrahedron* **1985**, *41*, 1093. Gustafson, K.; Andersen, R. J. *Tetrahedron* **1985**, *41*, 1101.
(4) Cimino, G.; Sodano, G.; Spinella, A.; Trivellone, E. *Tetrahedron Lett.* **1985**, *26*, 3389.
(5) Fasulo, G.; Izzillo, F.; Villani, G. *Boll. Malacologico* **1982**, *18*, 97.
(6) An analysis of the metabolites of *Haminea hydatis* will be reported in due course.
(7) Although it is now clear that these metabolites originate from *B. striata*, the name aglajne is maintained for these compounds to avoid confusion.
(8) Hochlowski, J. E.; Faulkner, D. J. *Tetrahedron Lett.* **1983**, *24*, 1917.
(9) Ksebati, M. B.; Schmitz, F. J. *J. Org. Chem.* **1985**, *50*, 5637.

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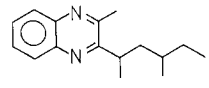
Table I. NMR Spectral Data of 1, 3, and 4

	^{13}C			^{1}H		
C	1[a]	3[b]	4[c]	1[a]	3[d]	4[d]
1	10.9	11.1 q[h]	11.1	0.86 t, 7.1	0.85 t, 7.4	0.85 t, 7.3
				1.33 m	1.34 m	1.35 m
2	29.5	29.5 t	29.5	1.10 m	1.09 m	1.08 m
3	32.4	32.2 d	32.3	1.28 m	1.26 m	1.24 m
4	19.4	19.4 q	19.4	0.85 d, 6.7	0.84 d, 6.6	0.84 d, 6.5
				1.72 ddd, 13.3, 5.7, 5.2	1.74 m	1.74 m
5	41.4	41.4 t	41.5	1.08 ddd, 13.3, 5.7, 5.2	1.07 m	1.06 m
6	37.1	36.7 d	36.8	3.34 m	3.33 m	3.34 m
7	18.5	18.7 q	18.7	1.04 d, 6.9	1.04 d, 6.8	1.04 d, 6.5
8	206.0	206.5 s	206.6			
9	135.7	135.1 s	135.3			
10	11.9	11.9 q	11.9	1.85 d, 1.2	1.84 d, 1.0	1.85 br s
11	142.9	144.0 d	143.9	6.43 d, 9.2	6.43 d, 9.3	6.44 d, 8.7
12	33.2	33.1 d	33.2	3.60 m	3.56 m	3.58 m
13	20.3	20.6 q	20.6	1.19 d, 6.7	1.17 d, 6.7	1.19 d 6.8
14	138.4	135.0 q	135.3	5.54 d, 9.0	5.35 d, 9.0	5.38 d, 9.0
15	132.0	131.6 s	131.6			
16	16.5	16.5[e] q	16.1[i]	1.92 d, 0.7	1.83 br s	1.87 br s
17	143.6	138.1 d	138.7	6.97 s	6.04 s	6.10 s
18	135.2	127.3 s	126.8			
19	13.2	16.9[e] q	16.9[i]	1.96 d, 1.0	2.03 d, 1.1	2.05 d, 1.1
20	199.4	165.4 s	158.6			
21	55.0	110.1[f] s	117.9	4.27 q, 7.0		
22	13.8	10.3[g] q	12.0	1.33 d, 7.0	2.05 s	1.86 s
23	207.6	165.5 s	181.4			
24	33.4	108.9[f] s	99.4	2.47 dq, 18.0, 7.2		
				2.39 dq, 18.0, 7.2		
25	7.7	11.9[g] q	6.9	1.03 t, 7.2	2.00 s	2.02 s
26		168.5 s	161.9			
OMe		60.2 q	55.3		3.83 s	3.96 s

[a] Data from ref 4. [b] Assignments made by ^{1}H–^{13}C heteronuclear COSY and by comparison with 1. [c] Assignments made by comparison with 1, 3, and model compounds (ref 9). [d] Assignments made by homonuclear decouplings and ^{1}H–^{1}H COSY. [e,f,g,i] Assignments with identical superscripts may be interchanged. [h] By DEPT sequence.

fragment from C-1 to C-11, thus leading to the complete assignment of the depicted structures. The ^{13}C chemical shift values of the vinyl methyl groups also established the *E* geometry of the double bonds.[10]

Furthermore a chemical proof was also achieved. Ozonolysis of aglajne-1 (1) and trapping of the resulting α-diketones with *o*-phenylenediamine afforded two quinoxaline derivatives **6** and **7** whose structures were easily



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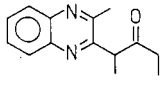

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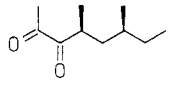

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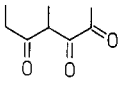

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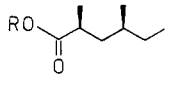


10, R=H
11, R=CH_3

determined on spectral grounds. **6** derives from the reaction of *o*-phenylenediamine with the α-diketone **8** arising from breaking of the 9–11 bond of aglajne-1; similarly breaking of the 17–18 bond gives rise to the α-diketone **9** and hence to the quinoxaline **7**.

Ozonolysis of aglajne-3 and similar workup resulted in the isolation of **6**, having a ^{1}H NMR spectrum and rotation identical with that of the same compound coming from the ozonolysis of aglajne-1. This result further confirms the structure of aglajne-3 (**2**) and establishes that the relative and absolute stereochemistry at C-3 and C-6 should be the same as in aglajne-1.

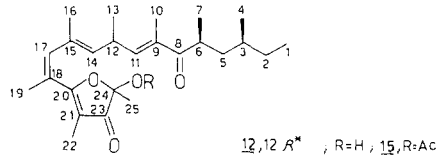
The relative and absolute stereochemistry at C-3 and C-6 was established as follows. The ozonolysis mixture of aglajne-1 (**1**), containing the α-diketone **8**, was treated with H_3IO_6[14] and the resulting 2,4-dimethylhexanoic acid (**10**) was isolated as its methyl ester (**11**) after treatment with ethereal CH_2N_2 and silica gel chromatography. The methyl ester **11** showed the same GLC retention time as well as the same ^{1}H NMR spectrum as methyl (2*S*,4*S*)-dimethylhexanoate synthesized as described by Odham,[11] which was readily distinguished from the 2*R*,4*S* diastereomer[11] which has a higher t_R on GLC and a different chemical shift value for the CH_3-2 in the ^{1}H NMR spectrum. The above evidence establishes the relative stereochemistry at C-2 and C-4 in **11**; furthermore **11** displayed a positive optical rotation, as the synthetic sample, and hence has the 2*S*,4*S* absolute configuration. From these data the *S*,*S* absolute configuration at the corresponding carbons (C-3 and C-6) in aglajne-1 (**1**) and -3 (**2**) was inferred.

The furanones **12**, **13**, and **14** were isolated as a single fraction after SiO_2 column chromatography exhibiting a single UV adsorbing spot on TLC using various eluents. They could not be separated by HPLC.

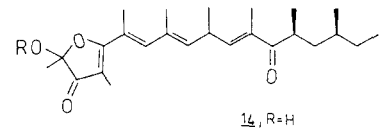
The ^{1}H NMR spectrum indicated that the mixture consisted of three compounds whose relative ratio changed in the extracts coming from the different collections of *B. striata*. In fact, the olefinic region of the ^{1}H NMR spec-

(10) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, 1972; pp 406–408.

(11) Odham, G. *Ark. Kemi* **1967**, *26*, 367.
(12) Capon, R. J.; Faulkner, D. J. *J. Org. Chem.* **1984**, *49*, 2506.
(13) Mareci, T. H.; Freeman, R. *J. Magn. Reson.* **1983**, *51*, 531.
(14) Jackson, E. L. *Org. React.* **1966**, 341.



12,12 R* ; R=H ; 15, R=Ac
13,12 S* ; R=H ; 16, R=Ac



14, R=H
17, R=Ac

trum of the mixture showed three sets of signals, each consisting of three different protons. One set showed signals at δ 6.74 (s), 6.42 (d, J = 8.9 Hz), and 5.49 (d, J = 8.9 Hz), later attributed to aglajne-2 (**14**), while the other two sets showed overlapped signals at δ 6.31 (doublets), 6.23 (singlets), and 5.36 (doublets), later attributed to compounds **12** and **13**. The ratio between the signals due to **12** and **13** was always 1:1, while the ratio between these latter taken altogether and those due to aglajne-2 (**14**) varied in the three collections of *B. striata* ranging from 0.7:1 in the October 1985 collection to the trace amount of **14** in the September 1985 collection.

The mixture of furanones **12**, **13**, and **14** from the October 1985 collection was acetylated, yielding three monoacetates (**15**, **16**, and **17**) which were readily separated by preparative TLC. The molecular formula was established to be $C_{27}H_{40}O_5$ for all compounds by HRMS. The ^{13}C NMR data (Table II) suggested the presence in these molecules of a 2-acetoxy-2,3-dihydrofuran-3-one ring, previously encountered in polypropionate metabolites from the pulmonate *Siphonaria lessoni*.[12]

The ^{13}C and ^1H NMR data of aglajne-2 acetate (**17**) for the portion of the molecule from C-1 to C-11 are practically identical with those of aglajne-1 (Table II), thus elucidating the structure of this part of the molecule.

The assignments were made by extensive use of conventional and 2D NMR techniques, including DEPT, ^1H–^1H COSY, ^1H–^{13}C heteronuclear COSY through direct and long range couplings, and proton double quantum coherence. This latter technique[13] was particularly useful because it gave a spectrum, without the presence of strong diagonal peaks, unlike its counterpart COSY, facilitating the detection of couplings in crowded regions. In Figure 1 the ^1H–^1H COSY and the proton double quantum coherence spectra of the δ 0.7–1.8 region of aglajne-2 acetate (**17**) are reported for comparison. The connectivity of the diene to the furanone was established by the ^1H–^{13}C heteronuclear long range COSY (Table III). It was in fact observed that the C-20 quaternary carbon ($\delta_{^{13}C}$ 178.4) was correlated with both the C-19 and C-22 methyl protons. On the other hand the C-17 carbon ($\delta_{^{13}C}$ 140.0) showed long range correlations with the C-16 and C-19 methyl protons, ruling out possible alternative structures.

As far as the stereochemistry is concerned, the E geometry of the double bonds was secured by the ^{13}C chemical shift values of the vinyl methyls,[10] while the stereochemistry at C-3, C-6, C-12, and C-24 remains to be determined. However, the relative stereochemistry at C-3 and C-6 should be the same as in aglajne-1 on consideration of the close similarity of the ^1H and ^{13}C chemical shift values on these and surrounding centers (Table II).

Aglajne-2 acetate (**17**) slowly isomerized on standing to a 1:1 mixture of two acetates (**15** and **16**) showing mass fragmentation virtually identical with that of **17**. Particularly indicative was the presence in the mass spectra of

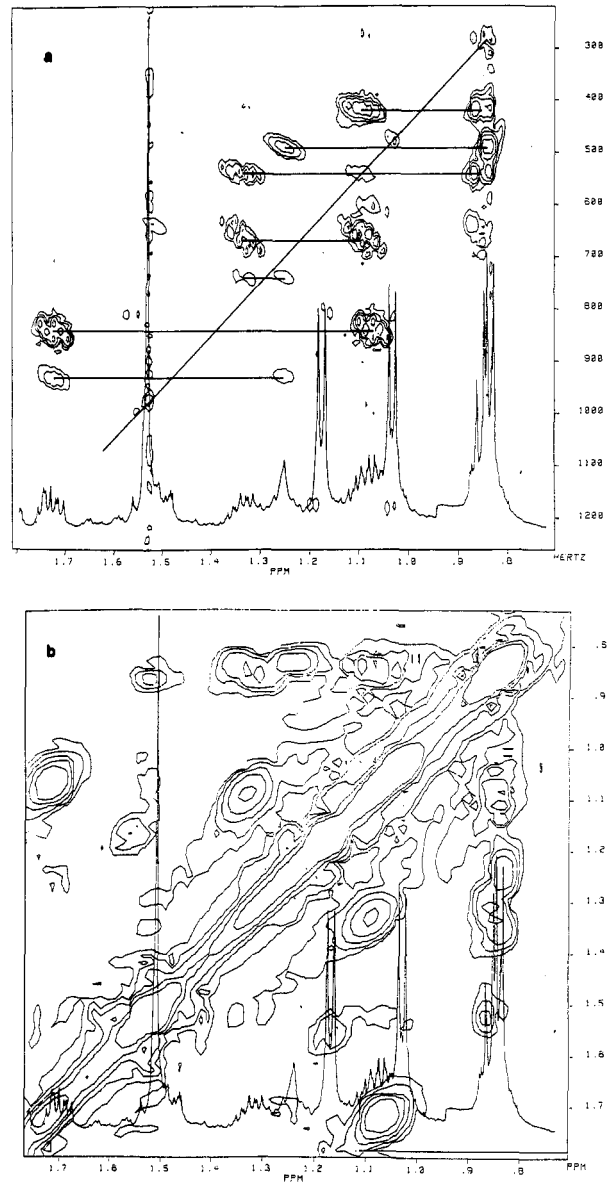


Figure 1. Upfield region of proton two-dimensional and conventional (superimposed) NMR spectra of aglajne-2 acetate (**17**): (a) proton double-quantum 2D-NMR; the pairs of coupled proton signals, joined by a horizontal line, are equidistant from the diagonal. (b) Proton–proton correlated 2D-NMR (COSY-45). A solution of 15 mg of **17** in $CDCl_3$ was used. Both the two-dimensional spectra were acquired as 256 × 1024 data points and transformed the using sine bell function in both dimensions.

15, **16**, and **17** of three major ions at m/z 195 ($C_{13}H_{23}O$), 196 ($C_{13}H_{24}O$), and 207 ($C_{12}H_{15}O_3$), which were interpreted as arising from the cleavage of the C-12,C-14 bond.

^1H and ^{13}C NMR data (Table II) indicated that the part of the molecule from C-1 to C-11 was unaffected by the transformation of **17** into **15** and **16**. The resonances were assigned by use of decoupling experiments, ^1H–^1H COSY, and ^1H–^{13}C heteronuclear COSY.

Furthermore, the ^{13}C NMR spectra indicated the presence in both **15** and **16** of a double bond with Z geometry. In fact two methyl groups resonating at δ 21.4 and 21.3, absent in the spectrum of **17**, were found in the ^{13}C spectra of **15** and **16**, respectively.

The ^1H–^{13}C heteronuclear COSY showed that these methyl groups were correlated to the methyl protons which in the ^1H NMR spectra were long range coupled to the internal proton of the diene system, hence establishing the Z geometry of the 17–18 double bond.

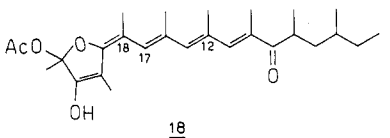
Table II. NMR Spectral Data of 15, 16, and 17

C		^{13}C			^{1}H		
		15[a]	16[a]	17[b]	15[c]	16[c]	17[d]
1	q[e]	11.1	11.1	11.1	0.82 t, 7.3	0.83 t, 7.2	0.85 t, 7.4
					1.28 m	1.32 m	1.32 m
2	t	29.6	29.5	29.4	1.06 m	1.07 m	1.09 m
3	d	32.3	32.3	32.3	1.24 m	1.24 m	1.24 m
4	q	19.3	19.4	19.4	0.81 d, 6.7	0.82 d, 6.6	0.83 d, 6.6
					1.67 m	1.70 m	1.70 m
5	t	41.7	41.6	41.4	1.04 m	1.05 m	1.05 m
6	d	36.3	36.7	36.7	3.31 m	3.29 m	3.32 m
7	q	18.7	18.7	18.7	1.02 d, 6.8	1.01 d, 6.7	1.03 d, 6.8
8	s	207.1	206.5	206.5			
9	s	134.8	135.2	135.3			
10	q	11.7	11.9	11.9	1.81 d, 1.2	1.79 d, 1.2	1.84 d, 1.2
11	d	144.0	144.0	143.6	6.53 d, 9.3	6.41 d, 9.5	6.41 d, 9.2
12	d	33.5	33.4	33.3	3.48 m	3.47 m	3.55 m
13	q	20.1	20.5	20.5	1.07 d, 6.6	1.11 d, 6.7	1.16 d, 6.7
14	d	135.0	135.6	137.2	5.44 d, 9.1	5.44 d, 8.9	5.45 d, 9.0
15	s	131.6	131.9	131.8			
16	q	15.8	15.5	16.8	1.73 d, 1.2	1.74 d, 1.2	1.88 d, 1.4
17	d	139.2	139.0	140.0	6.19 s	6.22 s	6.65 s
18	s	123.8	123.7	125.6			
19	q	21.3	21.4[f]	15.2	1.93 d, 1.7	1.94 d, 1.7	2.08 d, 1.6
20	s	179.8	179.9	178.4			
21	s	109.7	110.0	108.7			
22	q	6.7	6.6	7.7	1.48 s	1.53[h] s	1.91 s
23	s	199.8	n.d.[g]	200.7			
24	s	100.1	100.2	99.8			
25	q	21.5	21.5[f]	21.5	1.53 s	1.56[h] s	1.52 s
C=O	s	167.3	167.3	167.7			
CH_3	q	20.5	20.1	20.6	2.08 s	2.08 s	2.07 s

[a] Assignments made by ^{1}H–^{13}C heteronuclear COSY. [b] Assignments made by ^{1}H–^{13}C heteronuclear COSY through direct and long range couplings. [c] Assignments made by homonuclear decouplings and ^{1}H–^{1}H COSY. [d] Assignments made by homonuclear decouplings, ^{1}H–^{1}H COSY, and proton double quantum coherence. [e] By DEPT sequence. [f,h] Assignments with identical superscripts may be interchanged. [g] Not detected.

This finding establishes the major difference between **17** and the **15**–**16** couple.

As far as the difference(s) between **15** and **16** is concerned, since the gross structure is clearly the same, the distinguishing feature(s) could only reside in the stereochemistry of the C-12 or C-24 chiral centers, having established the similarity of the C-1–C-11 portion in **15**, **16**, and **17**. Epimerization at C-24 during the transformation of **17** into **15** and **16** should be ruled out since inversion at this center requires the opening of the furanone ring and loss of the acetyl group. It was therefore concluded that the transformation of **17** into **15** and **16** involves epimerization at C-12. This hypothesis is not unlikely: it could be imagined that the transformation of **17** into **15** and **16** takes place through the enol **18**; the restoration of the



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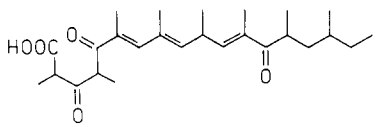

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ketonic form implies epimerization at C-12 and leads to the formation of a 17–18 double bond with *Z* geometry.

The above hypothesis is supported by the fact that **17** was transformed into **15** and **16** by mild base treatment. When aglajne-2 acetate (**17**) was treated with 1% $NaHCO_3$ in MeOH at room temperature small amounts of **15** and **16** were formed together with a mixture of **12** and **13**.

The facile transformation of **17** into **15** and **16** implies that compounds **12** and **13** should be regarded as artifacts originating from aglajne-2 (**14**) during the extraction and purification procedures. The fact that **12** and **13** were invariably found in a 1:1 ratio supports this view.

Table III. Long Range ^{1}H–^{13}C Couplings in Aglajne-2 Acetate (17)[a]

protons	carbon atoms correlated
1.84 (H-10)	135.3 (C-9), 143.6 (C-11)
1.88 (H-16)	137.2 (C-14), 131.8 (C-15), 140.0 (C-17)
2.08 (H-19)	140.0 (C-17), 125.6 (C-18), 178.4 (C-20)
1.91 (H-22)	178.4 (C-20), 108.7 (C-21), 200.7 (C-23)
1.52 (H-25)	99.8 (C-24), 200.7 (C-23)
2.07 (acetyl protons)	167.7 (acetyl carbonyl)

[a] Correlations observed in an experiment of ^{1}H–^{13}C heteronuclear COSY via long range couplings. The pulse sequences used were those supplied in the instrument manufacturer's software package; a *J* value of 7 Hz was used.

Aglajne-1 (**1**), -2 (**14**), and -3 (**2**) could be considered from a biogenetic point of view to originate from a common polypropionate precursor **19** which could lead to aglajne-1 by decarboxylation, to aglajne-3 by lactonization, and to aglajne-2 by oxidative decarboxylation followed by ketalization.

Experimental Section[15]

Extraction and Isolation Procedures. Specimens of *Bulla*

(15) ^{1}H NMR spectra were recorded at 500 MHz and ^{13}C NMR spectra at 125.8 MHz, all in $CDCl_3$, on a Bruker WM500 spectrometer; chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Mass spectra were obtained on AEI MS-30, MS-50, and MS-902 instruments. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. UV spectra were obtained on a Shimadzu Bausch & Lomb, Spectronic 210 spectrometer. IR spectra were recorded with a Perkin-Elmer Infracord 257 instrument. GLC's were performed on a Carlo Erba Fractovap HRGC instrument, equipped with a 25 m × 0.32 mm i.d. OV-101 fused silica capillary column (Macherey-Nagel).

striata were collected in the Bay of Naples in September 1985 (94 animals), October 1985 (50), and February 1986 (40). The extracts always contained the same compounds although the relative ratio within them proved to be different. The extraction and isolation procedures for the October 1985 collection is reported.

Freshly collected molluscs (50 animals) were immersed for 2 h in acetone (400 mL) with occasional crushing. The acetone solution was filtered and the extraction was repeated two times with the same amount of solvent.

The filtered acetone solution was concentrated and the concentrate diluted with H_2O and extracted with ether (3 × 250 mL). The ether extracts were combined and evaporated to yield an oil (1.641 g) which was chromatographed on SiO_2 using $CHCl_3$–MeOH (98:2) as eluant.

Fractions containing aglajne-1 (310 mg), were rechromatographed on SiO_2 using light petroleum ether–diethyl ether (15:5) to afford pure aglajne-1 (1; 146 mg). A fraction containing the mixture (177 mg) of aglajne-2 (14) and the furanones 12 and 13 was purified by flash chromatography on SiO_2 using light petroleum ether (7:3) as eluant, affording the mixture of 12, 13, and 14 (52 mg). The fraction (60 mg) containing aglajne-3 (2) was rechromatographed on SiO_2 (flash chromatography), yielding pure 2 (11 mg).

Mixture of aglajne-2 and furanones 12 and 13: UV (MeOH) 305, 234; ^1H NMR ($CDCl_3$) δ 6.74 (s), 6.42 (d, J = 8.9 Hz), 6.31 (overlapping doublets), 6.23 (s), 5.49 (d, J = 8.9 Hz), 5.39 (overlapping doublets), 3.58 (m), 3.46 (m), 3.32 (m), 3.26 (m), 2.11 (s), 1.98 (s), 1.89 (s), 1.85 (s), 1.56 (s), 1.55 (s), 1.43 (s), 1.41 (s); MS, m/z 402 (M^+), 207, 196, 195.

The mixture of 12, 13, and 14 (52 mg) was acetylated with Ac_2O (0.5 mL) and pyridine (1 mL) overnight at room temperature. The solvents were evaporated under a stream of N_2 and the residue was purified by semipreparative TLC (0.5 mm; light petroleum ether–diethyl ether, 7:3), yielding, in order of increasing polarity, 15 (10 mg), 16 (12 mg), and aglajne-2 acetate (17; 16 mg).

Compound 15: $[\alpha]^{20}_D$ +125° (c 0.5; $CHCl_3$); UV λ_{max} (CH_3OH) 309 (ϵ 7800), 230 (18700) nm; IR ($CHCl_3$) 1765, 1715, 1660, 1615 cm^{-1}; NMR data, Table II; MS, m/z 444.2879 (M^+; $C_{27}H_{40}O_5$ requires 444.2876), 384, 341, 313, 285, 257, 249, 207, 196, 195 (base peak), 179.

Compound 16: $[\alpha]^{20}_D$ +65° (c 0.2, $CHCl_3$); UV λ_{max} (CH_3OH) 309 (ϵ 7600), 230 (15500) nm; IR ($CHCl_3$) 1765, 1713, 1661, 1605 cm^{-1}; NMR data, Table II; MS, m/z 444.2903 (M^+; $C_{27}H_{40}O_5$ requires 444.2876), 384, 369, 359, 341, 313, 285, 257, 249, 221, 207, 196, 195 (base peak), 179.

Aglajne-2 acetate (17): $[\alpha]^{20}_D$ +46° (c 1.3; $CHCl_3$); UV λ_{max} (CH_3OH) 315 (ϵ 9100), 230 (15800) nm; IR ($CHCl_3$) 1765, 1705, 1660, 1605 cm^{-1}; NMR data, Table II; MS, m/z 444.2849 (M^+; $C_{27}H_{40}O_5$ requires 444.2876), 384, 341, 313, 285, 257, 207, 196, 195 (base peak), 179.

$NaHCO_3$ Treatment of Aglajne-2 Acetate (17). A 1% $NAHCO_3$ solution in MeOH (1 mL) was added to aglajne-2 acetate (10 mg). The reaction was monitored by TLC at 2-h intervals. After 4 h two spots appeared having the same R_f values as 15 and 16 when cochromatographed in various eluents, together with a more polar substance having the same R_f value as the non-acetylated product (12–14). After 16 h at room temperature the spots corresponding to 15, 16, and 17 disappeared and the solution was acidified with acetic acid, poured into water (10 mL), and extracted with ether (3 × 15 mL). The ethereal extract was evaporated and the residue was acetylated with Ac_2O (0.2 mL) in pyridine (0.5 mL) overnight at room temperature. The solution was evaporated under N_2 and the residue chromatographed on semipreparative TLC (light petroleum ether, 7:3), affording 15 (3 mg), 16 (3 mg), and aglajne-2 acetate (17, 2 mg), identified by their NMR spectra.

Aglajne-3 (2): $[\alpha]^{20}_D$ +105° (c 0.8; $CHCl_3$); UV λ_{max} (CH_3OH) 308 (ϵ 12400), 234 (19200); ^1H NMR δ ($CDCl_3$) 6.43 (1 H, d, J = 9.1 Hz), 6.01 (1 H, s), 5.33 (1 H, d, J = 8.7 Hz), 3.54 (1 H, m), 3.33 (1 H, m), 2.02 (3 H, s), 2.00 (3 H), 1.99 (3 H), 1.82 (6 H), 1.15 (3 H, d, J = 6.7 Hz), 1.02 (3 H, d, J = 6.8 Hz), 0.82 (3 H, t, J = 7.8 Hz), 0.81 (3 H, d, J = 7.0 Hz); MS, m/z 291, 284, 256, 196 (base peak), 195.

Methylation of Aglajne-3 (2) with CH_2N_2. To a solution of aglajne-3 (18 mg) in diethyl ether (5 mL) was added an excess of an ethereal solution of CH_2N_2. After 30 min at room temperature the excess of CH_2N_2 and the solvent were evaporated in vacuo and the residue was flash chromatographed on silica gel using light petroleum ether–diethyl ether (6:4) as eluant to give 5 mg of 3 and 4 mg of 4.

Pyrone 3: UV λ_{max} (CH_3OH) 312 (ϵ 9100), 230 (15600); IR ($CHCl_3$) 1705, 1665 cm^{-1}; NMR data, Table I; MS, m/z 428.2899 (M^+; $C_{27}H_{40}O_4$ requires 428.2926), 413, 368, 329, 301, 273, 247, 233 (base peak), 196, 195, 183.

Pyrone 4: UV λ_{max} (CH_3OH) 260 (ϵ 11100), 225 (18000); IR ($CHCl_3$) 1660 cm^{-1}; NMR data, Table I; MS, m/z 428.2915 (M^+; $C_{27}H_{40}O_4$ requires 428.2926), 413, 381, 371, 358, 329, 301, 269, 233 (base peak).

Ozonolysis of Aglajne-1 (1): Quinoxalines 6 and 7. A solution of aglajne-1 (1, 28 mg) in dichloromethane (10 mL) at −78 °C was treated with a stream of ozone for 5 min. The reaction mixture was stirred at −78 °C for a further 10 min after which zinc powder and acetic acid (2 mL) were added, and the mixture was allowed to warm to room temperature. The dichloromethane solution was partitioned with water and evaporated in vacuo. The residue was dissolved in pyridine (3 mL), *o*-phenylenediamine (20 mg) was added, and the mixture was refluxed for 2 h. The solvent was evaporated under a stream of N_2 and the residue was dissolved in $CHCl_3$ and purified by preparative TLC on silica gel using light petroleum ether diethyl ether (1:1) as eluant. Two UV-absorbing bands at R_f 0.9 and 0.5 were scraped from the silica gel and eluted with $CHCl_3$, yielding 6 mg of the quinoxaline 6 and 2 mg of the quinoxaline 7, respectively.

6: $[\alpha]^{20}_D$ +55° (c 0.6, $CHCl_3$); ^1H NMR δ ($CDCl_3$) 8.00 (2 H, m), 7.65 (2 H, m), 3.40 (1 H, m), 2.79 (3 H, s), 2.11 (1 H, m), 1.40 (2 H, m), 1.32 (3 H, d, J = 6.7 Hz), 1.26 (1 H, m), 1.13 (1 H, m), 0.87 (3 H, d, J = 6.3 Hz), 0.85 (3 H, t, J = 7.4 Hz); MS, m/z 242 (M^+), 227, 213, 185, 172, (base peak).

7: $[\alpha]^{20}_D$ −15° (c 0.2, $CHCl_3$); IR ($CHCl_3$) 1710 cm^{-1}; ^1H NMR δ ($CDCl_3$) 8.01 (2 H, m), 7.70 (2 H, m), 4.27 (1 H, q, J = 7.0 Hz), 2.78 (3 H, s), 2.46 (1 H, dq, J = 17.7 and 7.2 Hz), 2.38 (1 H, dq, J = 17.7 and 7.2 Hz), 1.60 (3 H, d, J = 7.0 Hz), 1.04 (3 H, t, J = 7.2 Hz); MS, m/z 228 (M^+), 172 (base peak).

Ozonolysis of Aglajne-3 (2). Treatment of a solution of 2 (40 mg) as described above yielded the quinoxaline 6 (4 mg), $[\alpha]^{20}_D$ +51° (c 0.4, $CHCl_3$). ^1H NMR and MS as above.

Synthesis of Methyl (2*S*,4*S*)-Dimethylhexanoate (11) and Methyl (2*R*,4*S*)-Dimethylhexanoate. Methyl (2*S*,4*S*)-dimethylhexanonate and methyl (2*R*,4*S*)-dimethylhexanoate were synthesized as described by Odham.[11] A mixture (2.8 g) of diastereomers was obtained starting from (+)-1-bromo-2-methylbutane (4.07 g; Aldrich) and diethyl methylmalonate (6.1 g) and following the previously outlined procedure;[11] 0.560 g of the mixture was chromatographed on a silica gel column (60 cm × 3 cm i.d.) with petroleum ether–diethyl ether as eluant (99:1), affording 25 mg of pure methyl (2*S*,4*S*)-dimethylhexanonate and 5 mg of methyl (2*R*,4*S*)-dimethylhexanoate, contaminated by ca. 20% of the 2*S*,4*S* diastereomer, together with several intermediate fractions containing mixtures of the two diastereomers in variable amounts.

Methyl (2*S*,4*S*)-dimethylhexanoate: $[\alpha]_D$ +28° (c 0.7, $CHCl_3$) (lit.[11] +32.2 ± 0.2); ^1H NMR δ ($CDCl_3$) 3.66 (s, OCH_3), 2.55 (m, H-2), 1.71 (m, H-4), 1.14 (d, J = 6.9 Hz, CH_3-2), 0.87 (d, J = 6.9 Hz, CH_3-4), 0.85 (t, J = 6.8 Hz, H_3-6); faster moving on GLC (OV-101; 40 °C).

Methyl (2*R*,4*S*)-dimethylhexanoate: ^1H NMR δ ($CDCl_3$) 3.66 (s, OCH_3), 2.5 (m, H-2), 1.7 (m, H-4), 1.11 (d, J = 6.9 Hz, CH_3-2), 0.87 (d, J = 6.9 Hz, CH_3-4), 0.85 (t, J = 6.8 Hz, H_3-6); slower moving on GLC (OV-101; 40 °C).

Ozonolysis of Aglajne-1 (1): Methyl (2*S*,4*S*)-Dimethylhexanoate (11). Ozonolysis of aglajne-1 (1, 37 mg) as above afforded a residue which was dissolved in CH_3OH (0.5 mL), and 0.5 mL of a 0.54 M H_3IO_6 aqueous solution was added.[14] After 2 days at room temperature the solution was made basic by addition of 20% aqueous KOH and extracted with diethyl ether. The aqueous phase was acidified with 1 N HCl and extracted with diethyl ether. Solvent removal afforded 5 mg of crude 10: ^1H NMR δ ($CDCl_3$) 2.57 (m, H-2), 1.73 (m, H-4), 1.18 (d, J = 6.9 Hz, CH_3-2), 0.88 (d, J = 6.6 Hz, CH_3-4), 0.87 (t, J = 7.0 Hz, H_3-6).

10 was methylated with ethereal CH_2N_2 and the resulting mixture was purified by silica gel chromatography on a pasteur

pipette affording ca. 1 mg of 11, α_D +0.030 ± 0.003° ($CHCl_3$); coeluting with synthetic methyl (2S,4S)-dimethylhexanoate on GLC; ^1H NMR identical with that of synthetic methyl (2S,4S)-dimethylhexanoate.

Acknowledgment. We gratefully acknowledge A. Crispino, S. Pacifico, and A. Trabucco for collecting *B. striata* and D. Ricciardi for technical assistance. Mass spectral data were provided by "Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli". The assistance of the staff is gratefully acknowledged.

Synthesis of a Novel Class of Peptides: Dilactam-Bridged Tetrapeptides

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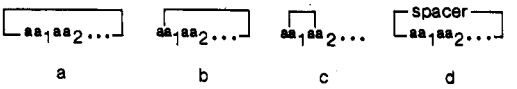
Received December 22, 1986

As model compounds for the study of constrained peptides, the following lactam-bridged derivatives were synthesized: Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe (I), Ac-L-Lys-L-Glu-L-Lys-L-Glu-NHMe (II), Ac-L-Lys-L-Glu-NHMe (III), Ac-L-Glu-L-Lys-D-Glu-D-Lys-NHMe (IV), Ac-L-Glu-L-Lys-L-Glu-L-Lys-NHMe (V), and Ac-L-Glu-L-Lys-NHMe (VI). Benzyloxycarbonyl and *tert*-butyloxycarbonyl groups were employed for amine protection and the benzyl and methyl esters for carboxyl protection. Coupling reactions were carried out by the use of active esters or through azide activation. Cyclization reactions were carried out by adding the active ester hydrochlorides into large volumes of pyridine at elevated temperatures. The cyclic intermediates were obtained in yields of 45–50%. Fragment condensation of the cyclic dipeptides yielded the corresponding dilactam-bridged tetrapeptides.

Introduction

The investigation of the secondary structures of proteins and polypeptides has been met with extensive interest in recent years.[1-5] However, because of the complexity of many naturally occurring molecules it has been necessary to study synthetic model compounds.[6-8] Such model compounds are usually designed to limit the degree of conformational freedom about the peptide backbone.[9,10] This limited flexibility, thus, facilitates the interpretation of spectroscopic data because of a reduced number of conformers.[11]

Model compounds have usually been constrained by either incorporating sterically hindered residues or via cyclization. Examples of the former type include N- and α-alkylated residues such as those found in N-methylated enkephalin analogues[12,13] and α-aminoisobutyric acid containing bradykinin[14] analogues, respectively. The other method for limiting conformational mobility of peptides is through cyclization. Intramolecular cyclizations can be carried out by coupling (a) backbone to backbone, (b) side chain to backbone, (c) side chain to side chain, or (d) by incorporating a "spacer" group within the ring:



The literature contains an extensive number of compounds belonging to classes a, b, and d. Examples of backbone to backbone, side chain to backbone, and spacer group containing peptides include gramicidin S[15] and valinomycin[16] analogues, enkephalin[17-19] analogues, and the AlaGly cyclic dipeptide[20] containing ϵ-aminocaproic acid, respectively. To date, very few synthetic compounds[21,22] containing side chain to side chain bridging have been reported.

(1) Hruby, V. J. *The Peptides*; Udenfriend, S., Meienhofer, J., Eds.; Academic Press: Orlando, 1985; Vol. 7, pp 1–14.
(2) Huang, D. H.; Walter, R.; Glickson, J. D.; Krishna, N. R. *Proc. Natl. Acad. Sci.* **1981**, *78*, 672–675.
(3) Llinas, M.; Horsley, W. J.; Klein, M. P. *J. Am. Chem. Soc.* **1976**, *98*, 7554–7558.
(4) Birktoft, J. J.; Blow, D. M. *J. Mol. Biol.* **1972**, *68*, 187–240.
(5) Quiocho, F. A.; Lipscomb, W. N. *Adv. Prot. Chem.* **1971**, *25*, 1–78.
(6) Deslauriers, R.; Evans, D. J.; Leach, S. J.; Meinwald, Y. C.; Minasian, E.; Nemethy, G.; Rae, I. D.; Scheraga, H. A.; Samarjai, R. L.f Stimson, E. R.; van Nispen, J. W.; Woody, R. W. *Macromolecules* **1981**, *14*, 985–996.
(7) Goodman, M.; Saltman, R. P. *Biopolymers* **1981**, *20*, 1929–1948.
(8) Howard, J. C.; Ali, A.; Scheraga, H. A.; Momany, F. A. *Macromol.* **1975**, *8*, 607–622.
(9) Veber, D. F.; Holly, F. W.; Nutt, R. F.; Bergstrand, S. J.; Brady, S. F.; Hirschman, R.; Glitzer, M. S.; Saperstein, R. *J. Am. Chem. Soc.* **1976**, *98*, 2367–2369.
(10) Veber, D. F.; Holly, F. W.; Nutt, R. F.; Bergstrand, S. J.; Brady, S. F.; Hirschman, R.; Glitzer, M. S.; Saperstein, R. *Nature (London)* **1979**, *280*, 512–514.
(11) Nutt, R. F.; Veber, D. F.; Saperstein, R. (1980) *J. Am. Chem. Soc.* **1980**, *102*, 6539–6545.
(12) Beddell, C. R.; Clark, R. B.; Hardy, G.; Lowe, L. A.; Ubatuba, F. B.; Vanei, J. R.; Wilkinson, S.; Chang, K. J.; Cuatrescasas, P.; Miller, R. J. *Proc. R. Soc. London, Ser. B* **1977**, *198*, 249–265.
(13) Roemer, D.; Buescher, H. H.; Hill, R. C.; Pless, J.; Bauer, W.; Cardinaux, F.; Closse, A.; Hauser, D.; Huguenin, R. *Nature (London)* **1977** *268*, 547–549.
(14) Vavrek, R. J.; Stewart, J. M. *Peptides* **1980**, *1*, 231–235.
(15) Ovchinnikov, Y. A.; Ivanov, V. T. *Tetrahedron* **1975**, *31*, 2177–2209.
(16) Gisin, B. F.; Merrifield, R. B.; Tosteson, D. C. *J. Am. Chem. Soc.* **1969**, *91*, 2691–2695.
(17) Berman, J. M.; Goodman, M. *Int. J. Pept. Prot. Res.* **1984**, *23*, 610–620.
(18) Berman, J. M.; Goodman, M.; Nguyen, T. M.-D.; Schiller, P. W. *Biochem. Biophys. Res. Commun.* **1983**, *115*, No. 3, 864–870.
(19) Richman, S. J.; Goodman, M.; Nguyen, T. M.-D.; Schiller, P. W. *Int. J. Pept. Prot. Res.* **1985**, *25*, 648–662.
(20) Nemethy, G.; McQuie, J. R.; Pottle, M. S.; Scheraga, H. A. *Macromolecules* **1981**, *14*, 975–985.
(21) Shiba, T.; Wakamiya, T.; Sukase, K.; Sano, A.; Shimbo, K.; Ueki, Y. *Biopolymers* **1986**, *25*, S11–S19.
(22) Kido, Y.; Hamakado, T.; Yoshida, T.; Anno, M.; Motokai, Y.; Wakamiya, T.; Shiba, T. *J. Antibiot.* **1983**, *36*, 1295–1299.

0022-3263/87/1952-5331$01.50/0