

AGLAJNE-1, A POLYPROPIONATE METABOLITE
FROM THE OPISTHOBRANCH MOLLUSK *AGLAJA DEPICTA*

DETERMINATION OF CARBON-CARBON CONNECTIVITY VIA LONG-RANGE ^1H - ^{13}C COUPLINGS

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Abstract. The structure of Aglajne-1, a polypropionate metabolite extracted from the opisthobranch *Aglaja depicta*, has been determined by extensive use of 2D-NMR and confirmed on chemical grounds.

As a part of a study of the defense allomones of opisthobranch mollusks¹, we have examined the chemical constituents of *Aglaja depicta* Renier 1807, (Gastropoda, Opisthobranchia)².

The diethyl ether solubles from the acetone extracts of the digestive glands of 37 specimens of *Aglaja depicta* were chromatographed on silica gel column (light petroleum-diethyl ether) to obtain three metabolites having a polypropionate carbon skeleton. Two metabolites were highly unstable and decomposed during the preliminary investigation, while to the less polar compound, named aglajne-1 (125 mg; oil; $[\alpha]_D^{+72}$, c 2.6, CHCl_3), structure 1 was assigned on the following evidence.

The molecular formula was defined as $\text{C}_{25}\text{H}_{40}\text{O}_3$ primarily on the basis of the ^{13}C -NMR data since 1 gives a very small molecular ion in the mass spectrum. The E.I. mass spectrum contained major fragments at m/z 193 (cleavage between C-12 and C-13) and at m/z 57 ($\text{CH}_3\text{-CH}_2\text{-C}\equiv\text{O}^+$); other structurally informative fragments were found at m/z 153 (cleavage between C-9 and C-10) and 137 (cleavage between C-16 and C-18). The IR spectrum contained bands for both saturated and unsaturated carbonyl groups (1710 and 1650 cm^{-1}). The UV spectrum (CH_3OH) showed maxima at 232 (ϵ 13,000) and 284 (ϵ 14,000) nm, this latter being shifted to 310 nm by addition of KOH. The ^{13}C -NMR spectrum (table 1B) indicated the presence of three carbonyl groups, two of which being assigned to a dienone and an enone system on the basis of the UV spectrum and ^1H -NMR data (table 1A). The third carbonyl group was assigned to a saturated ketone.

A combination of mono-dimensional and two-dimensional (2D)NMR methods allowed the assignments of the ^1H and ^{13}C chemical shifts and of the ^1H coupling constants. In particular δ - δ and J - δ ^1H correlations were provided by COSY and J -resolved experiments while the ^1H - ^{13}C correlations were established by a 2D-heteronuclear experiment. The results, reported in the table 1A-B, disclosed the presence

TABLE 1 - NMR data of aglajne -1^a

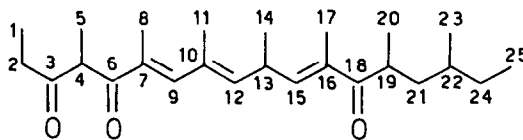
Position	A δ ¹ H	B δ ¹³ C	C Long-range ¹ H- ¹³ C couplings ^b
1	1.03 (t; J 7.2Hz)	7.7	-
2	2a 2.47(dq; J 18.0 and 7.2Hz) 2b 2.39(dq; J 18.0 and 7.2Hz)	33.4	H's-1 ^e
3	-	207.6	H's-1 ^e ; H's-5 ^e
4	4.27 (q; J 7.0Hz)	55.0	H's-5 ^e
5	1.33 (d; J 7.0Hz)	13.8	-
6	-	199.4	H's-8 ^{c,d} ; H-9 ^d ; H's-5 ^e
7	-	135.2	H's-8 ^{c,d}
8	1.96 (d; J 1Hz)	13.2	H-9 ^d
9	6.97 (s)	143.6	H's-11 ^d ; H's-8 ^d
10	-	132.0	H's-11 ^{c,d}
11	1.92 (d; J 0.7Hz)	16.5	-
12	5.54 (d; J 9.0Hz)	138.4	H's-11 ^d
13	3.60 (m)	33.2	H's-14 ^e
14	1.19 (d; J 6.7Hz)	20.3	-
15	6.43 (d; J 9.2Hz)	142.9	H's-14 ^{c,d} ; H's-17 ^{c,d}
16	-	135.7	H's-17 ^{c,d}
17	1.85 (d; J 1.2Hz)	11.9	H-15 ^d
18	-	206.0	H-15 ^d ; H's-17 ^{c,d} ; H's-20 ^e ; Ha-21 ^e
19	3.34 (m)	37.1	H's-20 ^e ; Ha-21 ^e
20	1.04 (d; 6.9Hz)	18.5	-
21	21a 1.72(ddd; J 13.3, 5.7, 5.2Hz) 21b 1.08(ddd; J 13.3, 5.7, 5.2Hz)	41.4	H's-20 ^e ; H's-23 ^e
22	1.28 (m)	32.4	H's-23 and/or H's-25 ^{e,d}
23	0.85 (d; J 6.7Hz)	19.4	Ha-21 ^e ; H-22 ^e ; H _b -21 and/or H _b -24 ^d
24	24a 1.33 (m) 24b 1.10 (m)	29.5	H's-23 and/or H's-25 ^{e,d}
25	0.86 (t; J 7.1Hz)	10.9	H-22 ^e ; H _b -24 ^e

^a 98 mg in 0.5 ml CDCl₃; Bruker WM 500 spectrometer; the pulse sequences used were those supplied in the instrument manufacturer's software package.

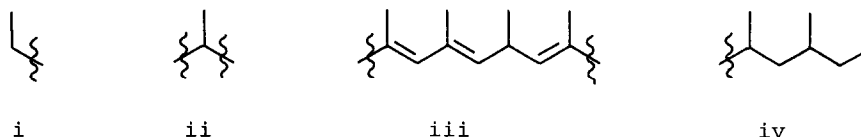
^b Protons long-range coupled with the carbon at the given position are reported.

^c Couplings observed using J=5Hz.

^d Couplings observed using J=10Hz.



of the partial structures i-iv, separated by carbonyl groups.



The complete carbon-carbon connectivity was deduced, as depicted in 1, from two ^1H - ^{13}C heteronuclear long range correlations using 5 and 10 Hz as J values (table 1C). It should be noted that the two experiments give complementary information which were of particular value for establishing the location of the carbonyl groups in the molecule.

Structure 1 implies, quite surprisingly, the presence of a completely non-enolized β -diketone moiety in aglajne-1. This unusual feature was confirmed in the following way. The shift induced by KOH from 284 to 310 nm in the UV spectrum primarily indicated the presence of an enolizable ketone. When the ^1H -NMR spectrum of aglajne-1 was recorded in NaOD-containing CD_3OD the C-4 proton and the C-5 methyl doublet disappeared and a newly formed vinyl methyl resonated as singlet at δ 1.99, indicating the presence in the solution of a completely enolized β -diketone. The chemical shifts of the olefinic protons of the conjugated diene system were also strongly affected by the addition of NaOD to the solution (fig.1), thus confirming that the β -diketone moiety was connected to the diene chromophore, while the remaining chemical shifts were practically unaffected.

Furthermore, reaction of 1 with $\text{NH}_2\text{OH}\cdot\text{HCl}$ (refluxing EtOH, NaOAc, 3h) gave two isomeric isoxazole derivatives, separated by preparative tlc (m/z 385, M^+ ; 286; 258; 190, base peak), whose ^1H -NMR data (fig. 2) confirm the attachment of the β -diketone to the diene system.

As far as the stereochemistry of 1 is concerned, the E geometry of the olefinic bonds was inferred from the ^{13}C -NMR chemical shifts of the vinyl methyl signals⁶, while the configuration at C-4, C-13, C-19 and C-22 remains to be determined.

"Polypropionate" metabolites have been previously isolated from few marine invertebrates, including sacoglossans^{3,4} and pulmonates⁵. However molecules so far isolated usually include γ -pyrone rings or cyclic emiketals in their structures, while aglajne-1 is a representative of acyclic polypropionates.

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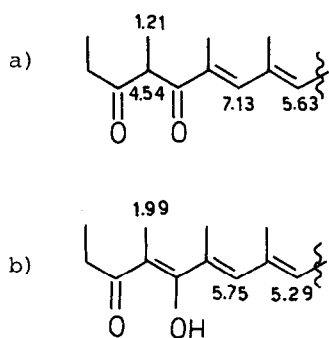


Fig. 1.- Relevant $^1\text{H-NMR}$ chemical shift values of a) aglajne-1 in CD_3OD , b) aglajne-1 in $\text{CD}_3\text{OD-NaOD}$.

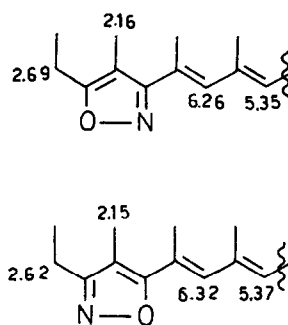


Fig. 2.- Relevant $^1\text{H-NMR}$ chemical shift values (CDCl_3) of the two isoxazoles obtained from aglajne-1; the set of values assigned to the two regioisomers may be mutually interchanged.

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