Tetrahedron Letters No. 23, pp 2017 - 2020, 1978. 0040-4039/78/0601-2017$02.00/0

THE STRUCTURES OF AMINO-ACID CONJUGATES OF BONELLIN DERIVED FROM THE MARINE ECHUROID BONELLIA VIRIDIS

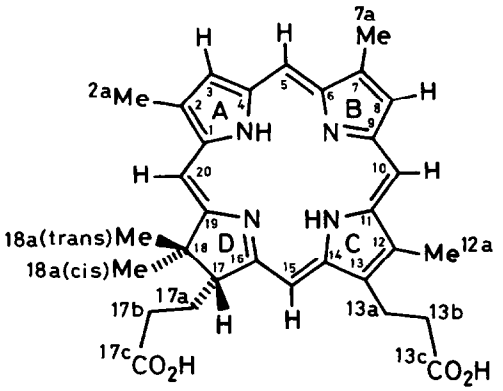
Andrew Pelter,[*a] Anthony Abela-Medici,[b] James A. Ballantine,[a] Victor Ferrito,[b] Stephen Ford,[a] Victor Jaccarini,[b] Alexander F. Psaila.[a,b]

(a) Chemistry Department, University College of Swansea, Swansea SA2 8PP, U.K.
(b) University of Malta, Msida, Malta

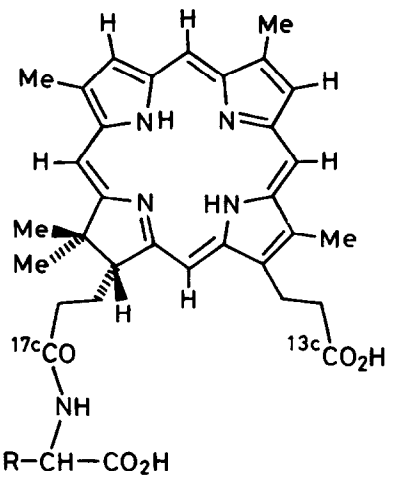
(Received in UK 22 March 1978; accepted for publication 13 April 1978)

SUMMARY A series of compounds in which amino-acids are conjugated to the C-17c carbonyl group of bonellin have been obtained from the body wall of Bonellia viridis.

We have previously shown that bonellin, the major physiologically active pigment of the probosces of the marine echuroid B. viridis has the structure (1).[1,2]



(1)



(2)

Although extracts from the body wall of the animal had the same absorption spectrum as bonellin it was noted that the larvae were rather specifically attracted to the probosces on which they settled and from which they removed the pigment. Hence the body walls and viscera of the animals were examined for pigments, which

were compared, by h.p.l.c. of their methyl esters, with the correspondingly treated extract from probosces. It was rapidly established that a second peak was present in all extracts, it being particularly abundant in that from the body walls (Table 1). No other porphyrins or chlorins were evidenced.

TABLE 1

Distribution of Pigments in B. viridis

	No. of animals	Wt. of purified material (mg)	BDME %	Peak 2 %
Body wall	20	57	50	50
Probosces	20	77	98	2
Viscera	20	0.5	71	29

Repeated column chromatography on Kieselgel G of the methylated extract gave crystalline material corresponding to peak 2 of better than 95% purity. The visible spectrum of this material was identical to that of BDME (bonellin dimethyl ester). Mass spectrometry showed the presence of a pair of homologues with molecular ions at m/e 667.3732 $\pm$ 0.0033 ($C_{39}H_{49}N_5O_5$) and m/e 653.3574 $\pm$ 0.0033 ($C_{38}H_{47}N_5O_5$). These peaks could correspond to methylated leucine and valine conjugates of bonellin.

Accordingly peak 2 components were hydrolysed with 6N HCl and the water soluble amino-acid hydrochlorides subjected to amino-acid analysis. The results (Table 2) indicate that there are four major components, with other as yet unidentified components accounting for only ca. 4% of the whole. The majority of the material is in the form of the valine, followed by the isoleucine conjugates. The tetrapyrrolic product from the acid hydrolysis was methylated and shown to be BDME. ^{1}H and ^{13}C n.m.r. spectra are completely in accord with the presence of an unmodified bonellin nucleus in the compounds comprising peak 2.

The mass spectrum of peak 2 contained no ions corresponding to di- or tri-peptides, confirming that in this extract all the amino-acid units correspond to different mono amino-acid conjugates of bonellin. The configuration of the major amino-acids was determined by g.l.c. of the N-pentafluoropropionylamino-acid-(-)-3-methyl-2-butyl esters.[3] The analysis, which was carried out on a 25m. glass capillary PLOT column coated with SE-30 (45,000 plate efficiency),

also independently confirmed the proportions and identities of the amino-acids present.[*]

TABLE 2

Amino-acid Composition of Bonellin Conjugates

Amino-acid	% of mixture	Configuration
Valine	62.7	L-
Isoleucine	23.0	L-
Leucine	5.9	L-
Alloisoleucine	4.0	D-

As the amino-acid conjugate esters (AACE) had the same absorption spectrum as BDME, which was produced from the acid hydrolysis, it was surmised that the amino-acid units must be attached through either or both of the two carboxyl groups. The mass and ^{1}H n.m.r. spectra gave information about the point of attachment.

In the mass spectrum an intense ion at m/e 467.2447 $\pm$ 0.0023 ($C_{29}H_{31}N_4O_2$) is evidenced. This results from total loss of a propionyl-amino-acid side chain ($-CH_2CH_2CONHCHRCO_2Me$) from the molecular ion. It has previously been observed[4] that in chlorins there is the complete loss of the C-17 propionate side chain from the saturated C-17 carbon atom. This loss is not evidenced in the methyl esters of porphyrins which instead undergo a 'benzylic' cleavage with loss of $-CH_2CO_2Me$. In the mass spectrum of AACE there was no significant 'benzylic' loss of $-CH_2CONHCHRCO_2Me$ to give an ion at m/e 481 corresponding to cleavage of amino-acid conjugate on the C-13 propionate side chain. Hence the amino acids are attached only at C-17c, as in (2).

This result is in accord with the ^{1}H n.m.r. of AACE. The signals due to the C-13 propionate side chain are unaffected by peptide formation, whereas the signals due to the protons on C-17a and C-17b were both broadened and modified in shape. Therefore the mono-peptide conjugates of bonellin are

[*] We thank Dr. R.J. Laub and Mr. K. Williams of the Chemistry Department, Swansea, for technical assistance with this analysis.

represented by (2). The mode of attachment of the peptide is very unusual for porphyrin type molecules, though somewhat reminiscent of vitamin B_{12}. We leave aside for a separate publication consideration of the physiological significance of the gross differences between the proportions of bonellin and its mono-peptide conjugates in the various portions of B.viridis.

Whilst the work was in progress we were informed by Dr. G. Prota[x] (University of Naples) that he has examined the body wall of B.viridis obtained from the sea near Naples. He has isolated the 17C-isoleucine conjugate of bonellin as the main component (ca. 93%) of the mixture, a result in contrast to ours, and he has named this compound neobonellin[5] in a talk on the subject. In view of our results which show that this particular amino-acid conjugate has no special significance, as compared say with the valine conjugate which is the main component of our mixtures, we suggest that the term neobonellin is inappropriate and that it should not be used.

One of us (A.F.P.) thanks the I.U.C. for financial help and one of us (S.F.) acknowledges the award of an S.R.C. studentship.

REFERENCES

1. A. Pelter, J. A. Ballantine, V. Ferrito, V. Jaccarini, A. F. Psaila, and P. T. Schembri, J.C.S. Chem. Comm., 1976, 999-1000.
2. A. Pelter, J. A. Ballantine, P. Murray-Rust, V. Ferrito, and A. F. Psaila, submitted to Tetrahedron Letters.
3. W. A. Konig, W. Rahn, and T. Eyem, J. Chromatogr. 1977, 133, 141-6.
4. A. H. Jackson, G. W. Kenner, K. H. Smith, R. T. Aplin, H. Budzikiewicz, and C. Djerassi, Tetrahedron, 1965, 21, 2913.
5. G. Prota, "IX Congresso della Societa' Italiana di Biologia Marina", Ischia, May 1977.

---

[x] We thank Dr. G. Prota for telling us of his results prior to publication.