Pergamon

*Tetrahedron Letters*, Vol. 38, No. 36, pp. 6327-6330, 1997

0040-4039/97 $17.00 + 0.00

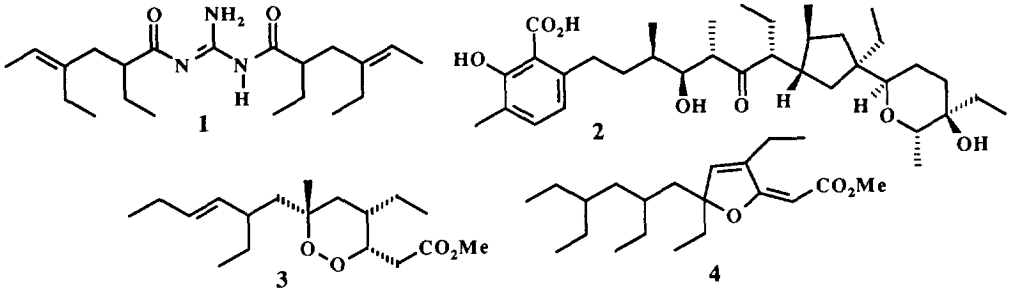
PII: S0040-4039(97)01455-X

# Evidence for the Incorporation of Intact Butyrate Units in the Biosynthesis of Triophamine

Julia Kubanek and Raymond J. Andersen*
Departments of Chemistry and Oceanography-Earth and Ocean Sciences
University of British Columbia
Vancouver, B.C.
CANADA V6T 1Z1

**Abstract:** *Biosynthetic feeding experiments with [2,3-$^{13}C_2$]butyrate and [2,3-$^{13}C_2$]ethylmalonate have provided evidence for incorporation of two intact butyrate units in the acyl residues of triophamine (1), a metabolite of the dorid nudibranch Triopha catalinae.* © 1997 Elsevier Science Ltd.

Triophamine (**1**) is a unique diacylguanidine metabolite that has been isolated from skin extracts of the Eastern Pacific dorid nudibranch *Triopha catalinae*.[1] Recently, we have shown that triophamine is biosynthesized *de novo* by *T. catalinae* and that the two identical ten carbon acyl residues are derived from five intact acetate units.[2] The presence of two ethyl appendages in the acyl residues of triophamine suggested that acetate incorporation into these fragments might occur in a processive manner via intact butyrate units through the intermediacy of the putative polyketide building block ethyl malonate. Although ethyl branches derived from butyrate are only rarely encountered in polyketide biosynthesis, precedent can be found in the biosynthesis of the microbial metabolites lasalocid A (**2**) and monensin.[3] In addition to triophamine (**1**), there are numerous other marine natural products such as plakortin (**3**)[4] isolated from the sponge *Plakortis halichondrioides* and the methyl ester **4** isolated from the sponge *Cladocroce incurvata*[5] whose biogenesis would also appear to involve butyrate units. However, to date there has been no experimental demonstration in marine organisms of polyketide biosynthesis involving intact butyrate or ethylmalonate units.




A proposed polyketide biogenesis for the ten carbon acyl residues in triophamine (**1**) starting from an acetyl CoA unit followed by elongation with two ethyl malonate units is shown schematically in Figure 1. In this biogenetic proposal, there is the assumption that ethylmalonate is in turn generated from acetyl CoA and malonyl CoA in order to account for the labeling pattern shown in **1a** that resulted from earlier [1,2-$^{13}C_2$]acetate feeding experiments with *T. catalinae*.[2]

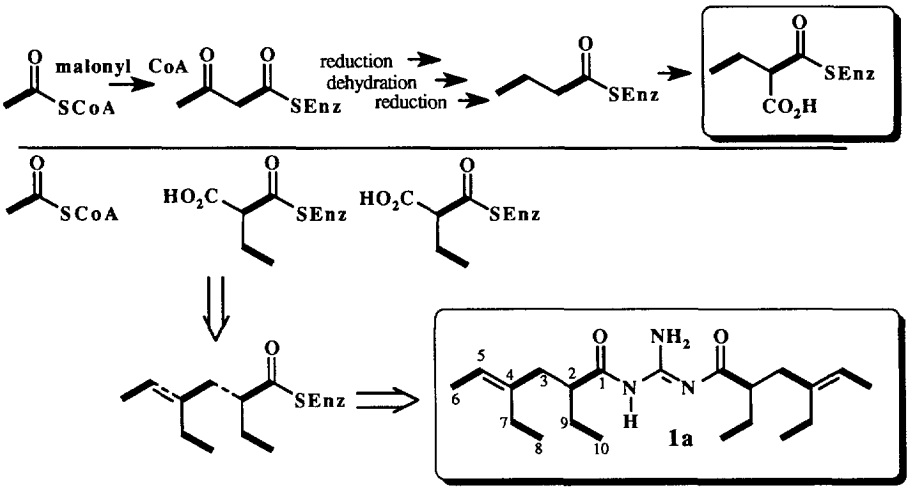



**Figure 1:** Proposed biogenesis for triophamine (1).

The objective of the current study was to demonstrate that the two four carbon fragments comprised of C3-C4-C7-C8 and C1-C2-C9-C10 in triophamine (**1**) are derived from intact butyrate units as required in a processive polyketide biosynthetic pathway involving the chain extension unit ethyl malonate as shown in detail in Figure 2. At the outset, it was anticipated that feeding experiments with isotopically labeled butyrate would be complicated by the catabolism of the butyrate to acetate followed by incorporation of the resulting labeled acetate

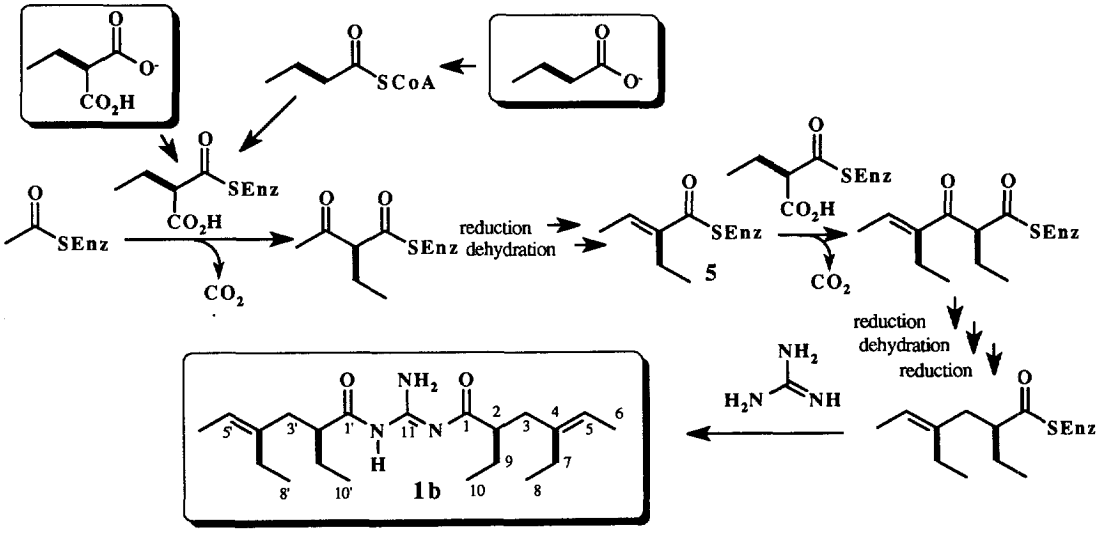



**Figure 2:** Details of a proposed processive polyketide biosynthesis of triophamine involving two butyrate units.

into triophamine.[6] Previous feeding experiments with acetate had also shown that only modest incorporation levels of labeled precursors could be expected in *T. catalinae*.[2] Therefore, the doubly labeled precursors [2,3-$^{13}C_2$]butyrate and [2,3-$^{13}C_2$]ethylmalonate were fed in the present experiments. These doubly labeled precursors offered two potential advantages: first, retention of the one bond $^{13}C/^{13}C$ scalar coupling in the triophamine (**1**) isolated from the feeding experiments would provide evidence that the C2-C3 bond in the butyrate equivalents had not been cleaved during incorporation, and second, the doublet resonances for enriched carbons in labeled samples would give visually convincing incorporation data that did not depend on measuring what were anticipated to be small peak height differences of singlet resonances resulting from low level incorporation of singly labeled precursors. An interesting side issue in the biogenesis of triophamine (**1**) concerned the pseudo-symmetry of the intermediate **5** (Figure 2), which is a 2,2-diethylacetic acid derivative. The processive biogenetic pathway proposed in Figure 2 predicts that [2,3-$^{13}C_2$]butyrate will label the saturated ethyl appendage (i.e. C4-C7) in triophamine (**1**). However, given the pseudo-symmetry in the carbon skeleton of intermediate **5**, the alternate labeling pattern (i.e. C4-C5) might also be possible. The butyrate incorporation experiments were expected to distinguish between the two possibilities.

Doubly labeled [2,3-$^{13}C_2$]ethylmalonate and [2,3-$^{13}C_2$]butyrate were synthesized from [2-$^{13}$C]diethyl malonate and [1-$^{13}$C]ethyl iodide as outlined in Figure 3.[7]

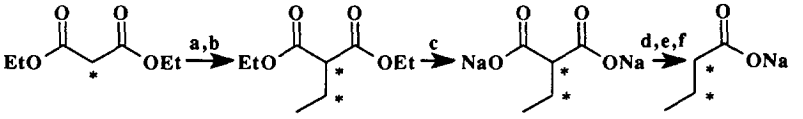



**Figure 3:** **a)** NaH, THF, 0°C, **b)** [1-$^{13}$C]EtI, reflux, 16h, **c)** NaOH, MeOH, reflux, 16h, **d)** 0.4N HCl, reflux, 72 h, **e)** titrate to neutral pH with $NaHCO_3$, **f)** lyophilize

*T. catalinae* feeding experiments with [2,3-$^{13}C_2$]ethylmalonate and [2,3-$^{13}C_2$]butyrate were conducted as previously described for [1,2-$^{13}C_2$]acetate.[2] Labeled precursors were dissolved in distilled water to give 0.55 M solutions that were administered to individual animals by injection through the dorsum at two times 16 h apart starting one day after collection of the animals from the field. After the second injection, the nudibranchs were left undisturbed in a running seawater aquarium for seven days at which time they were immersed whole in methanol. Labeled triophamine (**1**) was isolated from the methanol extracts using previously described chromatographic procedures.[1]

Examination of the $^{13}$C NMR spectrum of triophamine (**1b**) isolated from the [2,3-$^{13}C_2$]butyrate feeding experiment revealed that only four of the resonances, assigned to C2,2' (δ 50.1), C4,4' (δ 138.9), C7,7' (δ 22.6) and C9,9' (δ 25.4), showed flanking doublets indicating enrichment with doubly labeled butyrate (Figure 4). The average specific incorporation at the four labeled carbons was 0.17%. Analysis of the doublet coupling constants confirmed that C4,4' (J = 41 Hz) was coupled to C7,7' (J = 42 Hz) and that C2,2' (J = 33 Hz) was coupled to C9,9' (J= 34 Hz). The [2,3-$^{13}C_2$]ethylmalonate feeding experiment resulted in very low levels of incorporation. The C7,7' and C9,9' resonances in $^{13}$C NMR spectrum of the triophamine isolated from this feeding experiment showed very weak flanking doublets that were consistent with the predicted labeling pattern **1b**. However, because of the low levels of incorporation it was not possible to detect with

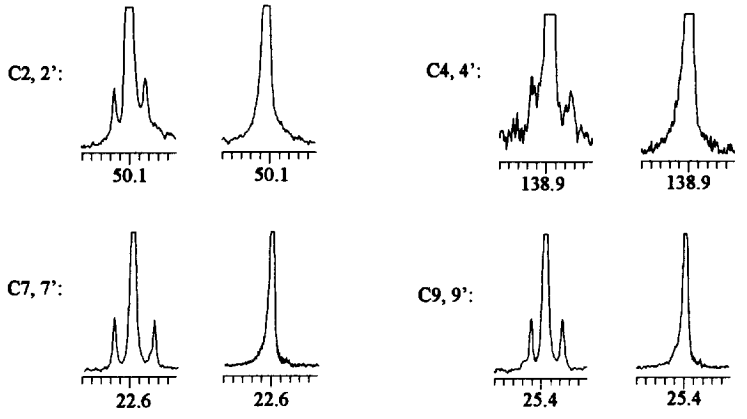



**Figure 4:** Selected resonances from the $^{13}C$ NMR spectrum of triophamine (**1b**) obtained from the [2,3-$^{13}C_2$]butyrate feeding experiment. All of the resonances have been normalized by plotting the central singlet to the same peak height and then truncating for ease of viewing. Left hand resonances in each pair are from the [2,3-$^{13}C_2$]butyrate acid feeding experiment; right hand resonances are from an unlabeled control sample.

certainty the presence of flanking doublets in either of the C2,2' or C4,4' resonances.

In summary, feeding [2,3-$^{13}C_2$]butyrate to *T. catalinae* has provided clear evidence for the intact incorporation of two butyrate units in the biosynthesis of triophamine (**1**). The incorporation pattern is consistent with a processive polyketide biosynthetic pathway in which butyrate is incorporated via the putative intermediate ethyl malonate as shown in Figure 2. [2,3-$^{13}C_2$]Ethyl malonate was not as effectively incorporated into triophamine in a companion feeding experiment. The present study represents the first experimental demonstration of polyketide biosynthesis involving butyrate units in a marine invertebrate. The chemical structures of numerous marine sponge metabolites such as **3** and **4** suggest that they have a similar biogenesis. Therefore, butyrate may be an important polyketide precursor in marine invertebrate biosynthesis.

## Acknowledgment

Financial support was provided by the Natural Sciences and Engineering Research Council of Canada. JK was supported by a NSERC '1967' Postgraduate Fellowship. The authors wish to thank Mike LeBlanc, Joelle Harris, and the staff of the Bamfield Marine Station for assistance with the collection and maintenance of *T. catalinae*.

## References

**1)** a) Gustafson, K.; Andersen, R.J. *J. Org. Chem.* **1982**, *47*, 2167, and b) Piers, E.; Chang, J.M.; Gustafson, K.; Andersen, R.J. *Can. J. Chem.*, **1984**, *62*, 1.
**2)** Graziani, E.I.; Andersen, R.J. *Chemical Communications* **1996**, 2377.
**3)** a) Westley, J.W.; Evans, R.H.; Harvey, G.; Pitcher, R.G.; Pruess, D.L.; Stempel, A.; Berger, J. *J. Antibiotics*, **1974**, *27*, 288, and b) L.E. Day, L.E.; Chamberlin, J.W.; Gordee, E.Z.; Chen, S.; Gorman, M.; Hamill, R.L.; Ness, T.; Weeks, R.E.; R. Stroshane, R. *Antimicrobial Agents and Chemotherapy* **1973**, *4*, 410.
**4)** Higgs, M.D.; Faulkner, D.J. *J. Org. Chem.* **1978**, *43*, 3454.
**5)** D'Auria, M.V.; Minale, L.; Riccio, R.; Zampella, A.; Debitus, C. *J. Nat. Prod.* **1993**, *56*, 418.
**6)** Hailes, H.C.; Jackson, C.M.; Leadlay, P.F.; Ley, S.V.; Staunton, J. *Tetrahedron Lett.* **1994**, *35*, 307.
**7)** All products gave NMR and high resolution MS data consistent with their assigned structures.

(*Received in USA* 10 *June* 1997; *accepted* 10 *July* 1997)