THE JOURNAL OF Organic Chemistry®

VOLUME 38, NUMBER 7

APRIL 6, 1973

Elucidation of Structure and Stereochemistry of Myriocin. A Novel Antifungal Antibiotic[1]

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Received November 2, 1972

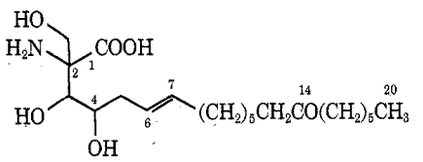
An antifungal principle myriocin was isolated from *Myriococcum albomyces*. The structure of this compound was elucidated using spectral and analytical data of its derivatives. The chemical reactions utilized in degradation work involved ozonolysis and periodic acid oxidation. Structure 1 was assigned to myriocin based on the available chemical data. The chemical and the physical evidence led to the stereochemical expression **28**.

In the course of a screening program directed toward the discovery of novel antimicrobial agents, antifungal activity was detected in the fermentation broth of *Myriococcum albomyces*, a thermophilic fungus of the ascomycete class. The active principle, responsible for the antifungal activity, was isolated from the broth of the microorganism grown in submerged culture, and was named myriocin.[2]

Myriocin (**1**) analyzed for $C_{21}H_{39}O_6N$ (401), having mp 180–181°, $[\alpha]^{24}D$ 10.3° (*c* 0.386, CH_3OH). An infrared spectrum (Nujol) showed broad hydroxylic absorption, 1702- and 1665-cm^{-1} bands, establishing the presence of a carbonyl. A mass spectrum showed no molecular ion peak. The highest ion peak was located at $M^+ - 18$ (*m/e* 383), with a base peak at $M^+ - (127 + 18)$ (*m/e* 256). The compound gave a positive ninhydrin test, suggesting the possible presence of an α-amino acid function. The antibiotic could not be satisfactorily esterified with diazomethane, and, owing to its insolubility in most organic solvents, including DMSO, no satisfactory nmr spectrum could be obtained.

When myriocin was heated in *tert*-amyl alcohol at reflux temperature overnight, it was dehydrated to give anhydromyriocin (**2**). This product was characterized by a new band at 1773 cm^{-1} in its infrared (Table III), suggesting a γ-lactone carbonyl. An nmr spectrum (220 MHz, Table II) showed a symmetrical multiplet, integrating for two vinylic protons, at δ 5.72.

Acetylation of myriocin (**1**) as well as anhydromyriocin (**2**) in pyridine–acetic anhydride yielded the triacetate **4**, corresponding to $C_{27}H_{43}O_8N$ (509). The formation of γ-lactone during the acetylation can be rationalized by the participation of the corresponding mixed anhydride of the acid followed by ring closure as shown in formula **5** (R = H). A mass spectrum exhibited a molecular ion M^+ (*m/e* 509), $M^+ - 60$ (*m/e* 449), $M^+ - 127$ (*m/e* 382), and $M^+ - (60 + 127)$ (*m/e* 322). The nmr spectrum of the triacetate **4** showed the signals which are listed in Table II. The interesting



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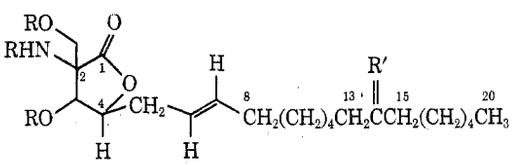
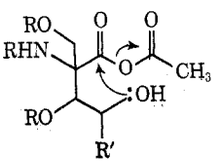


2, R = H; R′ = O

3, R = $COCH_3$; R′ = 

4, R = $COCH_3$; R′ = O



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(1) A preliminary account of this work was presented at the VIIth International Congress of Chemotherapy, Prague, Aug 1971.

(2) D. Kluepfel, J. F. Bagli, H. Baker, M. Charest, A. Kudelski, S. N. Sehgal, and C. Vézina, *J. Antibiot.*, **25**, 109 (1972).

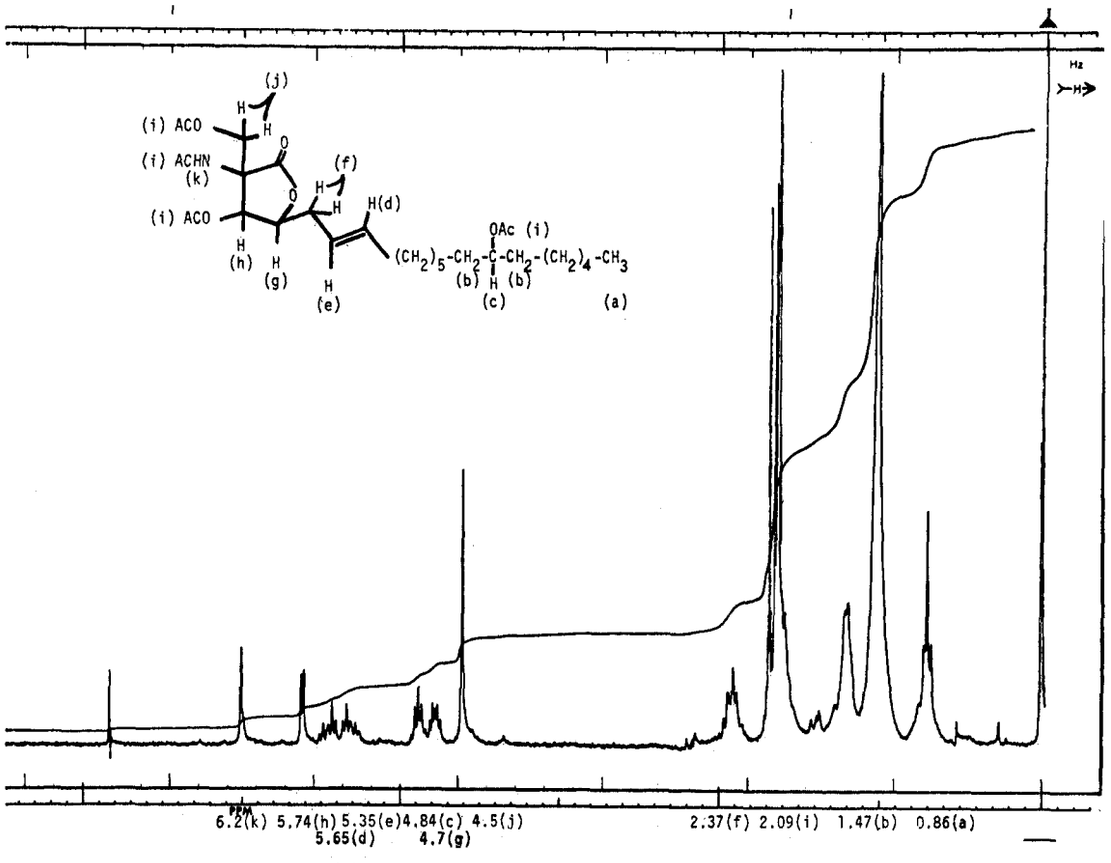


Figure 1.—Nmr (220 MHz) of tetraacetate **4**.

feature in the nmr of compound **4** was a cluster of signals integrating for six protons and appearing as a badly resolved triplet, centered at δ 2.4. Acetylation of a sodium borohydride reduction product of myriocin yielded a tetraacetate **3**, whose nmr spectrum showed a decreased intensity signal (integrating for 2 H) at δ 2.4. The acetylation of a product obtained from the catalytic hydrogenation of the parent antibiotic was characterized by the absence of vinylic proton peaks and by a signal at δ 2.4 now integrating for four protons. Finally, an acetylation, preceded by both catalytic as well as hydride reduction, yielded a product for which the peaks at δ 2.4 and 5.5 (vinylic protons) were completely absent while an appropriate increase in the number of protons at higher field was noted. These results are summarized in Table I.

TABLE I

NUMBER OF PROTONS CORRESPONDING TO THE SIGNALS AT δ 2.4 AND 5.5 IN THE NMR SPECTRA OF VARIOUS REACTION PRODUCTS

Experiment	δ 2.4 signal	δ 5.5 signal
1 —(1. Ac_2O)→ triacetate **4**	6 H	2 H
1 —(1. $NaBH_4$; 2. Ac_2O)→ tetraacetate **3**	2 H	2 H
1 —(1. cat./H_2; 2. Ac_2O)→ triacetate	4 H	
1 —(1. $NaBH_4$; 2. cat./H_2; 3. Ac_2O)→ tetraacetate		

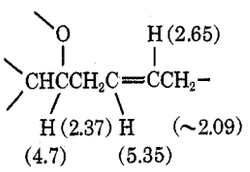
Assuming that the δ 2.4 multiplets represent the protons on carbon atoms α to a ketone, and those α to >C=C<, the above evidence clearly established the presence of $-CH_2COCH_2-$ and $CH_2HC{=}CH-$ moieties in the acetylation products. The 1702-cm^{-1} band present in the parent antibiotic is therefore attributable to a ketone. The above experiments suggested the downfield shift for one allylic methylene relative to the other, and this was indeed confirmed by a detailed analysis of the nmr (220 MHz) of the tetraacetate **3** (Figure 1).

The relevant signals of the nmr spectrum (220 MHz) of the tetraacetate **3** are listed in Table II. In addition to those listed, the spectrum exhibited a broad singlet at δ 1.47 which integrated for four protons, and a quintuplet at δ 4.84 attributable to a carbinolic proton. In a double-resonance experiment, irradiation at δ 1.47, the frequency of the former, reduced the quintuplet to a singlet. This experiment established the presence of the $-CH_2CH(OCOCH_3)CH_2-$ grouping, thus confirming the presence of the ketone and the nature of the substitution on carbons α,α' to the ketone (*vide supra*) in the parent antibiotic. The spectrum also showed a quintuplet at δ 2.37 (2 H), whose relationship to protons of chemical shift at δ 5.35 and 5.65 (vinylic, 2 H) and at 4.7 (1 H) was deduced from the double-resonance experiments as described below. Irradiation at δ 2.37 collapsed the quintuplet (4.7) to a poorly resolved doublet; at the same time, the 5.35 signal collapsed to a doublet. Furthermore, irradiation of the allylic signal at δ 2.09 (partially buried under the acetate peak) reduced the δ 5.65 multiplet to a doublet. The above experiments permit the following assignments and established the presence of the structural feature **6** in the tetraacetate **3**.

TABLE II
NMR DATA

Compd	NH	C-2 (CH_2OR)	C-3	C-4	C-6 and -7	C-20[a]
2		3.61 (q)	4.15 (d)	4.66 (m)	5.72 (m)	0.9
3	6.2 (s)	4.50 (s)	5.74 (d)	4.7 (q)	5.35, 5.65 (m)	0.86
4	6.3 (s)	4.51 (s)	5.79 (d)	4.74 (m)	5.5 (q)	0.88
8	6.46 (s)	4.48 (s)	5.70 (d)	5.24 (q)		
13		4.12 (q)	5.32 (d)	4.70 (m)	5.56 (m)	0.87
15	7.05 (s)	3.98 (s)	5.31 (d)	4.70 (m)	5.56 (m)	0.87
17	6.84	3.88	4.62 (m)[b]		5.56 (m)	0.88
18	6.59	4.43 (s)	4.57 (m)[b]		5.59 (m)	0.9
19		4.0 (q)	5.17 (d)	4.58 (m)	5.58 (q)	0.88
20		4.48 (q)	5.08 (d)	4.48[b]	5.58 (q)	0.90

[a] These signals always appeared as a badly resolved triplet. [b] C-4 signals in this case were overlapping with C-2 CH_2OH.



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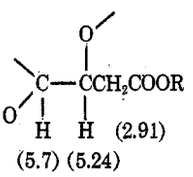

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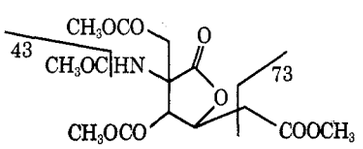

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The trans geometry of the double bond follows from (a) presence of a 950–970-cm^{-1} band in the ir spectra of the various derivatives (Table III) as well as of myriocin

TABLE III
IR DATA

Compd	Hydroxyl region	>C=O stretching region	Vinylic hydrogen bending
1	Broad	1702, 1665	962
2	3475	1775, 1705	975
4	3340[a]	1785, 1755, 1712,[b] 1665	950
3			
8	3400,[a] 3350[a]	1780–1725, 1675	
13	3375	1775, 1700, 1648, 1598	
15	3400	1775, 1700, 1635, 1595, 1509	

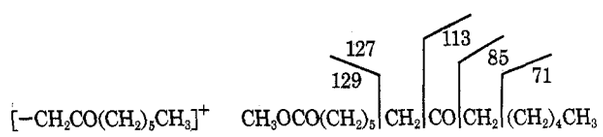
[a] N–H stretching. [b] The bands appeared as a shoulder on a broad absorption.

(1) and (b) J = 16 Hz in the nmr spectrum of tetraacetate **3**.

Ozonolysis of the triacetate **4**, followed by oxidative work-up with hydrogen peroxide and the esterification of the resulting acidic mixture, yielded two major products. A crystalline solid was obtained, whose elemental analysis led to the empirical formula $C_{14}H_{19}O_9N$. An ir spectrum showed a broad carbonyl 1760–1725 cm^{-1}, as well as a band at 1675 cm^{-1}. A mass spectrum showed a molecular ion M^+ (m/e 345), $M^+ - 43$ (m/e 302), and $M^+ - 73$ (m/e 272). An nmr spectrum (Figure 2) exhibited a doublet at δ 2.91 (2 H, J = 7 Hz), a quartet at 5.24 (1 H), and a doublet at 5.7 (1 H, J = 6 Hz). Irradiation at δ 2.91 led to the collapse of the δ 5.24 quartet to a doublet (J = 6 Hz). Conversely, the δ 2.91 doublet collapsed to a singlet when the quartet at 5.24 was irradiated. In addition, irradiation at δ 5.7 transformed the quartet to a triplet. These results are best accommodated by the partial formula **7** and the structure of the ozonolysis product may be expressed as **8**.

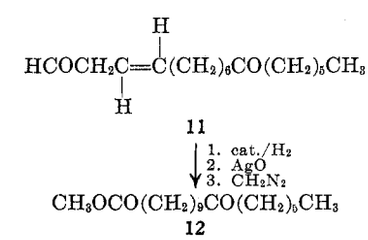
The second product of the ozonolysis appeared from its ir and nmr data to be a long-chain fatty acid ester. A mass spectrum showed a molecular ion at m/e 256, and a strong peak due to fragment m/e 129 ($M^+ - 127$). In the mass spectra of myriocin (**1**) and that of the triacetate **4** strong peaks were present for a fragment (*vide supra*) from the loss of mass 127. Considering the presence of a saturated ketone in a straight chain the m/e 127 can arise from a fragment $[C_8H_{15}O]^+$, which may be expanded to expression **9** for this fragment.

This, coupled with m/e 113, 85, and 71, led to the structure **10** for this product. The above assignment



was confirmed by comparison of the tlc, glc, and mass spectrum of this product with those of an authentic sample[3] of 8-ketotetradecanoic acid methyl ester.

Treatment of myriocin (**1**) with 4 equiv of periodic acid in a mixture of ether–water led to the isolation of an aldehyde in excellent yield. The ir of this product showed characteristic bands at 2710, 1710, and 970 cm^{-1} (vinylic proton bending). The nmr (100 MHz) exhibited a triplet at δ 9.65 and a multiplet at 3.09. The triplet above collapsed to a singlet when the decoupler frequency was applied at δ 3.09. The uv spectrum showed no absorption in the neutral medium; however, in the basic medium a band developed at 233 nm (ϵ 5100). This is attributable to a double bond shift from $\beta\gamma \rightarrow \alpha\beta$ position of a carbonyl. The aldehyde was assigned structure **11**. This assignment was un-



(3) F. L. Breusch and A. Kirkali, *Fette, Seifen, Anstrichm.*, **65**, 995 (1963).

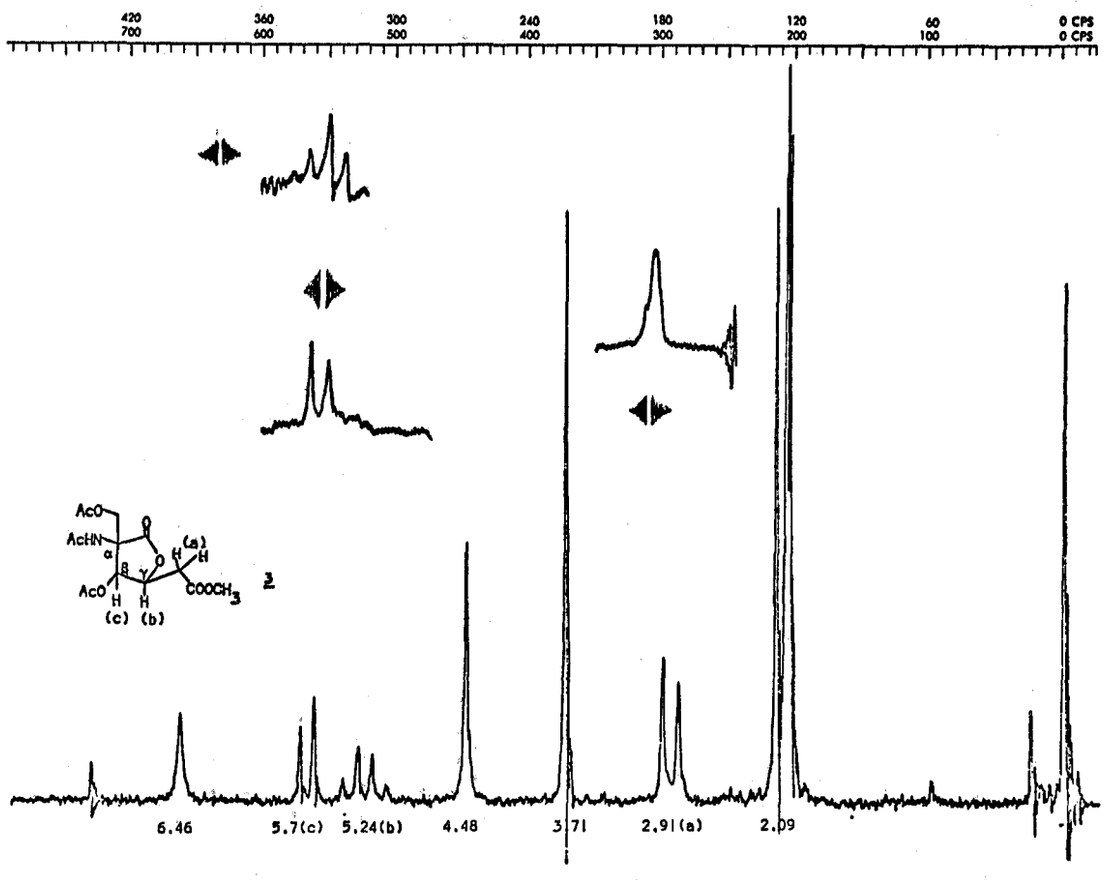


Figure 2.—Nmr (100 MHz) of the methyl ester **8**, showing the results of the spin decoupling between δ 5.7, 5.24, and 2.91 signals.

ambiguously established as follows. Catalytic reduction of the aldehyde **11**, followed by the silver oxide oxidation, yielded a saturated carboxylic acid. This was esterified with diazomethane to yield the corresponding methyl ester **12**. Both the acid and the methyl ester were found to be identical in all respects with an authentic sample[4] of 11-ketoheptadecanoic acid and its methyl ester.

The above experimental and analytical data are best accommodated in the expression **2** for anhydromyriocin and consequently the structure **1** for myriocin.

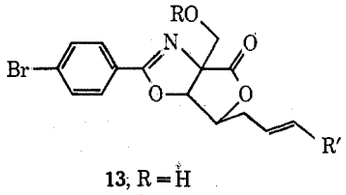
Stereochemistry.—Myriocin has three asymmetric centers, *viz.*, C-2, C-3, and C-4. The following experimental evidence led to the assignment of stereochemistry at these positions.

Treatment of myriocin with *p*-bromobenzoyl chloride in pyridine yielded a product homogeneous by tlc, which partially crystallized. The nmr spectra (Table II) of the crystals (minor product) and the oil (major product) were different, as shown below. It was evident from these spectra that they were both monobenzoates.

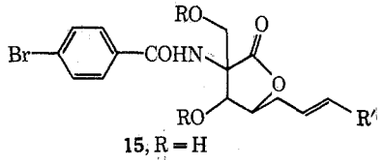
Nmr differences between two products of benzoylation

Benzoate	NH	C-2 CH_2OH (2 H)	Aromatic (4 H)
Oil	Absent	δ 4.1 (q)	δ 7.68 (q)
Solid	Present δ 7.05	δ 3.98 (s)	δ 7.60 (s)

Acetylation of the purified benzoates yielded two products. A major product was obtained whose nmr spectrum showed one acetyl methyl (δ 2.03), and its mass spectrum showed a molecular ion M^+ (*m/e* 590). In contrast, the spectrum of the minor component showed two acetyl groups (δ 2.08 and 1.88) and a molecular ion at *m/e* 650. Based on the above data, the major, oily benzoate and its acetate were assigned structure **13** and **14**, respectively. The minor benzoate and the corresponding acetate are therefore expressed as **15** and **16**.



13, R = H
14, R = $COCH_3$



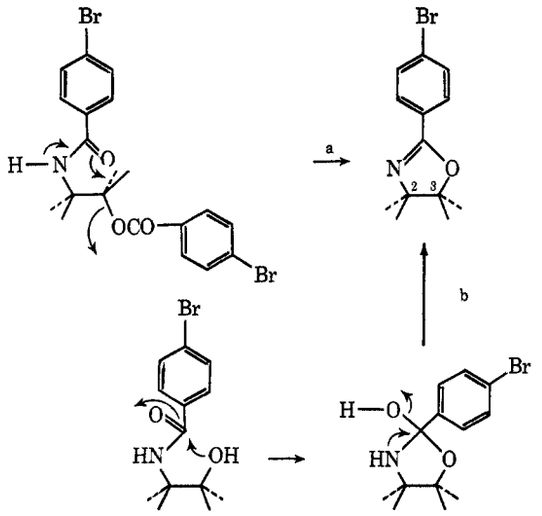
15, R = H
16, R = $COCH_3$

R' = $-(CH_2)_6CO(CH_2)_5CH_3$

Under the conditions of benzoylation (pyridine–*p*-bromobenzoyl chloride), myriocin is first transformed *via* the mixed anhydride of the type **5** leading to anhydromyriocin. This compound undergoes N-benzoylation to give the minor monobenzoate **15**. The formation of oxazoline **13** can result from the eventual benzoylation of the C-3 hydroxyl followed by nucleophilic

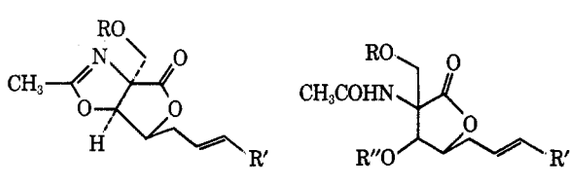
(4) F. L. Breusch and A. Kirkali, *Fette, Seifen, Anstrichm.*, **67**, 4 (1965).

participation of the *N*-benzoyl carbonyl and the ejection of -OCOPh-*p*-Br. Such a pathway to the generation of oxazolines is well documented.[5a]



The above mechanistic pathway (a) would imply trans stereochemistry of C_2N and C_3O in anhydromyriocin (2). An alternate pathway (b) involving the attack by oxygen electrons[5b] is unlikely under the basic conditions employed. The lack of benzoylation of the primary alcohol may be attributed to steric reasons.

Further evidence for the trans stereochemistry of C–N and C–O bonds at carbon atoms 2 and 3 is afforded by the acetylation of myriocin under conditions of selective N-acetylation[6] in methanol–acetic anhydride. This experiment led to the isolation of the two major and two minor products. Based on the ir (Table III) and the nmr (Table II) data the major products were assigned structures **17** and **18**, whereas the minor products were assigned structures **19** and **20**.

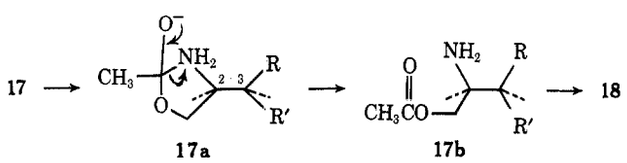


19, R = H
20, R = $COCH_3$

17, R = H; R″ = H
18, R = $COCH_3$; R″ = H
21, R = $COCH_3$; R″ = Ms

R′ = $-(CH_2)_6CO(CH_2)_5CH_3$

In contrast to the benzoylation described above, *N*-monoacetate **17** is now formed predominantly. The small tendency of the acetylation of the C-3 hydroxyl group followed by ring closure *via* pathway a described above explains the formation of oxazolines **19** and **20** as minor products. The second major product **18** can be rationalized through N → O acetyl migration *via* intermediate **17a** and **17b** followed by reacetylation[7] of the NH_2 group.



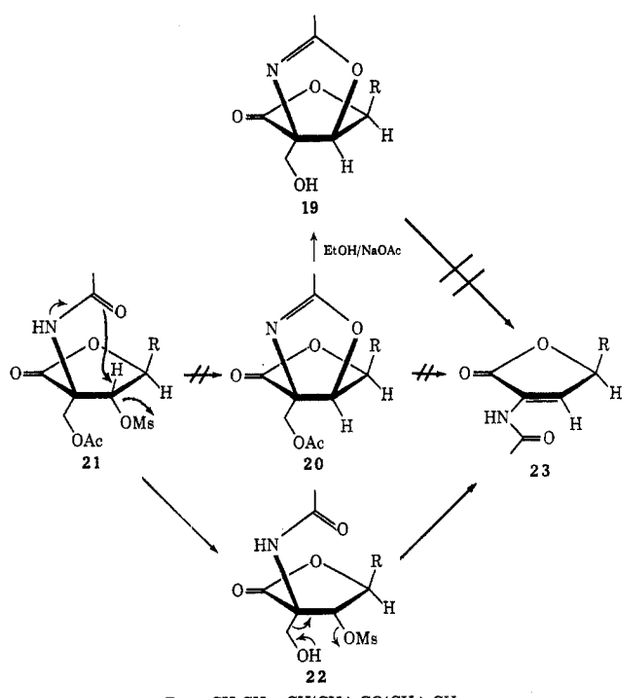
17a **17b**

If R in formula **17a** was a hydroxyl a similar N → O acetyl migration involving C-3 hydroxyl would lead to acetylation of this secondary hydroxyl group. The observation that no such C-3 acetate was formed and that the oxazolines **19** and **20** cannot be formed by pathway b under the conditions of the experiment suggests that the C-2–N and C-3–O bonds are trans to each other.

Therefore, the formation of oxazoline **13** as a major product (under conditions that promote both N- and O-esterification) and the oxazolines **19** and **20** as minor products (under conditions that promote preferential N-esterification) led us to infer that they are formed *via* the pathway suggested. Consequently the trans stereochemistry of the C–N and C–O bonds at C-2 and C-3 follows.

Mesylation of the diacetate **18** in methylene chloride–triethylamine[8] or in pyridine with methanesulfonyl chloride led to the formation of mesylate **21**. Treatment of mesylate **21** in ethanol and sodium acetate[5a] at reflux temperature overnight led in good yield to the isolation of a product that analyzed for $C_{22}H_{35}NO_4$.

The infrared spectrum showed a new band at 1742 cm^{-1} attributable to a butenolide with the concomitant loss of the saturated lactone band at 1775 cm^{-1}. The nmr spectrum exhibited a vinylic proton doublet (J = 2 Hz) at δ 7.4, and a doublet of triplets at 5.02 attributable to a carbinolic proton. The relationship between these two protons was established by double-resonance studies. The doublet at δ 7.4 collapsed to a singlet when observed during the irradiation at the resonance frequency of the proton at δ 5.02. Conversely, the doublet of triplets reduced to a triplet when observed



R = $-CH_2CH{=}CH(CH_2)_6CO(CH_2)_5CH_3$

(5) (a) L. Goodman, *Advan. Carbohyd. Chem.*, **22**, 109 (1967); (b) S. Konstas, I. Photaki, and L. Zervas, *Chem. Ber.*, **92**, 1288 (1954).

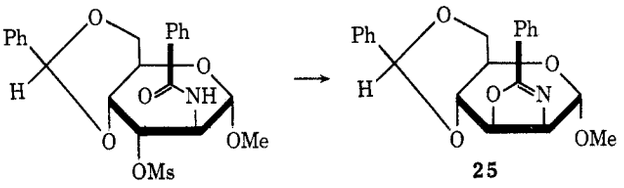
(6) Y. Inouye, K. Onodera, S. Kitaoka, and S. Hisano, *J. Amer. Chem. Soc.*, **78**, 4722 (1956).

(7) An alternative possibility of the formation of *N*,*N*-diacetyl compound as a precursor where one acetyl group migrates N → O cannot be discounted.

(8) R. K. Crossland and K. L. Servis, *J. Org. Chem.*, **35**, 3195 (1970).

during the irradiation at δ 7.4. This compound was assigned structure **23**.

It has been reported[9] that the α-D-altroside derivative **24** in refluxing ethanol and sodium acetate leads to oxazoline **25**. If one assumes a similar loss of the



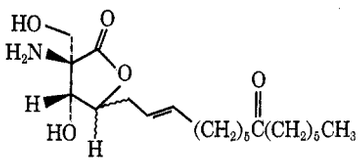
24 **25**

mesylate group in the genesis of compound **23** this would imply (a) trans stereochemistry of the methansulfonyl group relative to the –NHAc group and (b) that compound **20** is an intermediate in the formation of butenolide **23**.

However, when the oxazoline **20** was refluxed overnight in ethanol and sodium acetate, it led to the isolation of alcohol **19** as a major product. This experiment provides evidence that (1) under these conditions the methanolysis of the primary acetate occurs with ease and (2) alcohol **19** is not an intermediate in the formation of olefin **23**. It therefore follows that the mesylate **21** is hydrolyzed to the primary alcohol **22**, which undergoes a 1,3-diol cleavage to eliminate formaldehyde leading to the generation of the butenolide **23**.

Solvolytic[10] fragmentation of this type may or may not proceed *via* a concerted cyclic pathway. However, assuming (*vide supra*) the cis stereochemistry of the mesylate and the hydroxymethyl group in intermediate **22** a six-membered cyclic transition state leading to olefin **23** appears a probable pathway.

The above chemical evidence allows the expression of anhydromyriocin as shown in **26**.



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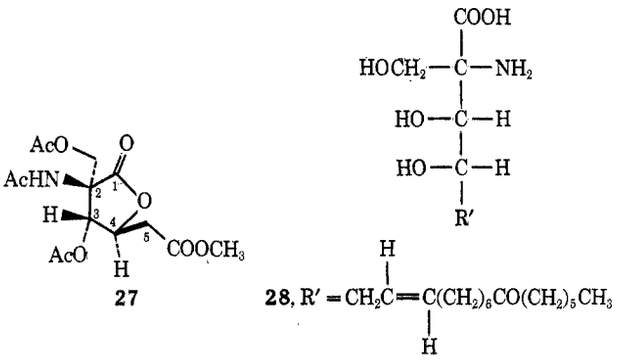
NOE Spectrum Analysis.—In order to provide direct evidence in support of the above assignments at C-2 and C-3, and to determine the stereochemistry of substituents at C-4, we have made use of the intramolecular proton nuclear Overhauser effect (NOE), which provides a sensitive means for determining relative internuclear distances.[11] The investigation of the methyl ester **8** revealed the following. Irradiation of the resonance frequency of the methylene singlet at C-5 led to a 20 ± 3% enhancement of the integrated intensity of the NH signal, and a 15 ± 5% increase in the area of the C-3 proton. Similar experiments between the C-3 and the C-4 protons were not possible owing to the close proximity of their chemical shift. A small NOE of *ca.* 5% was detected between the C-3 proton and the methylene group attached to C-2.

Since only a large NOE can yield meaningful results, the above data demonstrates unambiguously that the C-5 methylene and the NHAc proton are on the same side of the ring. The interpretation of the NOE between C-5 methylene and the proton at C-3 warrants further discussion. Recent results[12] on the derivatives of penicillin indicate that, for a given conformation of the five-membered ring, both cis and trans methyl groups led to a significant NOE for an adjacent proton (the NOE for cis methyl was greater than that for the trans methyl). In another conformation of the same group of compounds, only the cis methyl group led to a large NOE. Recent work[13] on acetonides and related compounds also suggests that in a five-membered ring a methyl group produces a significant increase in the area of the adjacent proton, on the same side of the molecule. It therefore follows that C-5 methylene and C-3 proton in ester **8** bear a cis relationship to each other as shown in **27**. Consequently, the opening of the lactone ring of the anhydromyriocin **26** would lead to myriocin,[14] the asymmetric centers of which can be represented as shown in the expression **28** or its mirror image.



27 **28**, R′ = $CH_2C(=CH_2)(CH_2)_6CO(CH_2)_5CH_3$

Experimental Section[15]

Myriocin (1).—The compound obtained from microbial fermentation was purified by repeated crystallizations from methanol, mp 180–181°, $[\alpha]^{24}D$ +10.3° (*c* 0.386, CH_3OH). The infrared spectrum (Table III) was recorded in KBr and showed broad hydroxylic absorption characteristic of a carboxylic acid.

Anal. Calcd for $C_{21}H_{39}O_6N$ (401): C, 62.81; H, 9.79; N, 3.49. Found: C, 62.95; H, 9.67; N, 3.32; $M^+ - 18$ (*m/e* 383), $M^+ - (127 + 18)$ (*m/e* 256).

Anhydromyriocin (2).—A solution of myriocin (1) (0.275 g) in *tert*-amyl alcohol (33 ml) was refluxed overnight. The solvent was removed and the residue (0.22 g) was purified by chromatography on silica gel (22 g) using 10% methanol–chloroform. A pure fraction recrystallized from chloroform–petroleum ether (bp 30–60°) to give lactone **2**, mp 76–77°, $[\alpha]^{24}D$ +33.4° (*c* 0.718, CH_3OH). The infrared and the nmr spectra are recorded in Tables III and II, respectively.

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(14) Since the submission of this paper, we have noted that thermozymocidin, a natural product [F. Aragozzini, *et al.*, *Experientia*, 881 (1972)] recently reported, appears to be identical with myriocin in its gross structure.

(15) The infrared spectra were recorded for solids in chloroform and for oils as film on a Perkin-Elmer model, and ultraviolet spectra were done in ethanol on a Unicam spectrophotometer. Unless otherwise mentioned, all routine nmr spectra were recorded on a 60-MHz Varian A-60A spectrometer. Decoupling experiments were carried out either at 100 MHz on a Jeol JNM-4H-100 instrument or at 220 MHz on a Varian HR-220, through the facilities of the Canadian 220 MHz NMR Centre, Sheriden Park, Ontario. The Merck silica gel (mesh 0.05–0.2 mm) was used for column chromatography. Organic extracts were dried over anhydrous magnesium sulfate, and the solvents were always removed under vacuum. Mass spectra were recorded on a Hitachi RMU-60 spectrometer.

Anal. Calcd for $C_{21}H_{37}O_5N$ (383): C, 65.76; H, 9.72; N, 3.65. Found: C, 65.79; H, 9.79; N, 3.40.

Anhydromyriocin hydrochloride was prepared by passing dry HCl gas in a solution of anhydromyriocin (0.3 g) in dry ether (37 ml) at 0°. After saturation the solvent was removed and the residue was crystallized from methanol–ether twice to give a pure sample (0.150 g), mp 180–185°.

Anal. Calcd for $C_{21}H_{38}NO_5Cl$: N, 3.35; Cl, 8.41. Found: N, 3.69; Cl, 8.41.

Acetylation of Myriocin. A. In Pyridine.—A sample of myriocin (132 mg) was acetylated with pyridine (2 ml) and acetic anhydride (2.8 ml) overnight at room temperature. The reaction mixture was worked up in the usual manner to yield a crude product (0.151 g). Purification by chromatography gave a sample of homogeneous triacetate **4** as an oil. The nmr and the ir are shown in Tables II and III.

Anal. Calcd for $C_{27}H_{43}O_8N$ (509): C, 63.63; H, 8.51; N, 2.75. Found: C, 63.63; H, 8.80; N, 2.91.

B. In Methanol.—To a suspension of myriocin (1) (10 g) in methanol (150 ml) at 65° was added acetic anhydride (200.3 ml). The mixture was stirred at that temperature for 30 min. The solvent was removed and the residue was flushed with methanol. The residue was taken in chloroform, washed with water, and dried and the solvent was removed to yield the crude mixture (11.5 g). This was chromatographed on silica gel (700 g) in 7% methanol–chloroform. A compound (3.29 g) was isolated from the later (71–94) fractions. Crystallization from methanol–ether gave pure *N*-acyl derivative **17** (3 g), mp 106–107°. An analytical sample from the same solvent had mp 106–107°.

Anal. Calcd for $C_{28}H_{39}O_6N$ (425): C, 64.91; H, 9.24; N, 3.29. Found: C, 64.84; H, 9.33; N, 3.08.

An ir spectrum showed absorptions at 3400 (broad band), 1773, 1705, and 1653 cm^{-1} (carbonyl region). The relevant nmr signals are listed in Table II.

Fractions 28–47 (5.4 g) were rechromatographed. The earlier fractions (13–20) of this chromatogram were pooled with the analogous fractions from the first purification to yield a product (0.75 g). Three crystallizations from ether–petroleum ether gave a pure sample, mp 72–73°. This was assigned structure **20**.

Anal. Calcd for $C_{25}H_{39}O_6N$ (449): C, 66.79; H, 8.75; N, 3.12. Found: C, 66.59; H, 8.86; N, 3.34.

An ir spectrum showed absorptions at 1778, 1750, 1705, and 1662 cm^{-1}. The nmr signals are listed in Table II.

Further elution gave a product (3.1 g) which slowly crystallized from ether–petroleum ether, mp 55–57°.

Anal. Calcd for $C_{25}H_{41}O_7N$ (467): C, 64.21; H, 8.84; N, 3.00. Found: C, 64.29; H, 8.92; N, 2.71.

An ir spectrum showed bands at 3300, 1778, 1750, 1705, and 1662 cm^{-1}. The nmr signals (Table II) and the above data led to the assignment of diacetate **18** for this product.

Finally, the later fractions (59–70) were pooled with similar material from the previous chromatogram and repurified to yield a product (0.58 g). Its structure **19** follows from the data below. Two crystallizations from ether–petroleum ether gave 0.35 g of product, mp 70–71°.

Anal. Calcd for $C_{23}H_{37}O_5N$ (407): C, 67.78; H, 9.15; N, 3.44. Found: C, 67.74; H, 9.32; N, 3.40.

An ir had broad absorption in the OH region, and carbonyl bonds at 1776, 1708, and 1660 cm^{-1}.

Reduction Experiments with Myriocin. A. Sodium Borohydride.—Myriocin (0.125 g) was dissolved in methanol (15 ml), and sodium borohydride (50 mg) was gradually added. After 20 min the reaction was quenched with saturated ammonium chloride solution (1.4 ml) and the product was extracted with chloroform to yield a residue (0.145 g). This was acetylated with pyridine–acetic anhydride to yield the tetraacetate **3**. An ir spectrum showed bands at 3400 (NH stretching), broad carbonyl absorption with peaks at 1775, 1748, 1700 and 1675, and a broad peak at 1225 cm^{-1} (sp^2 C–O stretching). The nmr signals are listed in Table II.

B. Catalytic Reduction.—Myriocin (0.1 g) was dissolved in methanol (12 ml) and hydrogenated in the presence of 5% palladium on charcoal (0.08 g). After filtration and removal of the solvent, a crude product (0.095 g) was obtained. This was acetylated in pyridine–acetic anhydride to yield the dihydro triacetate (0.057 g). An ir spectrum exhibited bands at 3400 (NH stretching), 1776, 1750, 1687 (broad, carbonyl), and 1212 cm^{-1} (ester). An nmr showed signals at δ 6.22 (1 H, s, NH), 5.76 (1 H, d, J = 5 Hz, carbinolic), 4.68 (1 H, m), 4.51 (2 H, s, carbinolic), 2.38 (4 H, m), 2.08, 2.03, 2.0 (s, 3 H each, acetyl methyl), 0.88 (3 H, t, terminal methyl).

C. Product of Borohydride and Catalytic Reduction.—A sample of myriocin (0.147 g) was reduced with sodium borohydride as described above. The product (0.15 g) was subjected to catalytic reduction to yield 0.16 g of tetrahydromyriocin. This was acetylated in the usual manner to yield a tetraacetate (0.085 g). An ir showed bands at 3410, 1750 (broad carbonyl absorption with shoulders at 1775 and 1723), 1680, and 1225 cm^{-1}. An nmr exhibited signals at δ 6.36 (1 H, s, NH), 5.82 (1 H, d, J = 5 Hz, carbinolic), 4.8 (1 H, m), 4.53 (2 H, s, carbinolic), 2.1 (12 H, m, acetyl methyl), and 0.88 (3 H, t, terminal methyl). Absence of signals at ~2.4 was conspicuous.

Ozonolysis of Acetate 4.—The acetate **4** (0.62 g) was dissolved in chloroform (15 ml) and ozonized for 1 hr at −50°. The solvent was removed and the crude ozonide was subjected to oxidation. It was dissolved in acetic acid (40 ml), and 30% hydrogen peroxide (10 ml) was added to it. The mixture was kept at ~80° (bath temperature) for 24 hr. The solvent was removed and the residue was esterified with diazomethane. Chromatography of the product on silica gel and elution with 5% ethyl acetate–benzene gave a product (0.15 g). Purification of this product *via* base-catalyzed hydrolysis, isolation of the acid, and reesterification gave a compound identical in its mass spectrum and glc (12 ft, 5.1% XE60, T_c 215°, retention time 6.2 min) with 8-ketotetradecanoic acid methyl ester.[3] An ir spectrum showed bands at 1737 and 1712 cm^{-1}. Further elution with 70% ethyl acetate–benzene yielded a product (0.338 g) which was recrystallized from methanol–ether once, to yield a sample of lactone **8** (0.225 g), mp 174–175°. An analytical sample obtained from the same solvent had mp 174–175°.

Anal. Calcd for $C_{14}H_{19}O_9N$ (345.3): C, 48.69; H, 5.55; N, 4.06. Found: C, 48.78; H, 5.54; N, 4.55.

The nmr and ir data are listed in Tables II and III, respectively.

Periodic Acid Oxidation of Myriocin.—Myriocin (1) (0.2 g) was suspended in ether (3 ml). To this suspension was added with vigorous stirring a solution of periodic acid (0.5 g) in water (2 ml). The reaction mixture was stirred for 15 min. The reaction mixture was diluted with ether, washed with a solution of thiosulfate and with water, and dried, and the solvent was removed. The crude product (0.132 g) was triturated and washed with ice cold petroleum ether to yield a product (0.102 g) homogenous by tlc. The ir showed an aldehydic proton stretching at 2710, a carbonyl band at 1710, and vinylic proton bending at 970 cm^{-1}. A uv spectrum showed no characteristic band in neutral medium. In basic medium a band at 223 nm (ϵ 5100) developed. The nmr showed the following signals: δ 0.88 (3 H, t, terminal $-CH_3$), 3.09 (2 H, m, $-CH_2$ α to aldehydic carbonyl), 5.53 (2 H, m, vinylic protons), 9.65 (1 H, t, HC=O).

Transformations of the Aldehyde 11.—A sample (0.309 g) of aldehyde **11** was dissolved in methanol (19 ml) and hydrogenated in presence of 5% palladium on charcoal (0.150 g). The product (0.301 g) was isolated in the usual manner. An ir showed absence of 970 cm^{-1} (vinylic proton bending). The above product was dissolved in ethanol (7.5 ml). To this solution was added a solution of silver nitrate (0.75 ml, 50%), followed by addition of sodium hydroxide solution (0.75 ml, 23%). The mixture was stirred overnight at room temperature and then filtered through Celite. The ethanol was removed and the residue was taken in ether. The ether extract was washed with sodium hydroxide (10%). The aqueous liquor was acidified with dilute hydrochloric acid, the acid thus obtained was extracted with ether and dried, and the solvent was removed to yield a crude product (0.163 g). This was pooled with the acid (0.07 g) obtained from another experiment. The mixture was esterified with diazomethane to yield crude ester (0.22 g) and was put on a silicic acid column (70 g) and eluted with 2% ethyl acetate–benzene to give pure ester **12** (0.09 g). A glc of this ester showed identical retention time with that of methyl 11-ketoheptadecanoate.[4] A sample (0.082 g) of the above ester was hydrolyzed with methanolic sodium hydroxide to yield the corresponding acid (0.062 g). A crystallization from ether–petroleum ether gave crystals (0.05 g), mp 78–79°, mmp with authentic 11-ketoheptadecanoic acid 78–79° (reported[4] 78–79°).

Benzoylation of Myriocin (I).—To a solution of myriocin (0.45 g) in dry pyridine (10 ml) was added *p*-bromobenzoyl chloride (1.8 g). The reaction mixture was heated to 100° for 24 hr. The mixture was cooled and diluted with methanol and the solvent was removed. The residue was taken in ether, washed with

hydrochloric acid (3%), sodium bicarbonate, and water, and dried and the solvent was removed. The residue (0.575 g) was passed through a column of silica gel (50 g) to yield a product (0.290 g) homogenous by tlc. On keeping, some crystals appeared which were separated with ice-cold hexane. Based on the nmr (Table II) and ir data (Table III) the crystalline benzoate was assigned structure **15**, and the oily benzoate was assigned structure **13**. The acetylation of the mixture of benzoates (0.210 g) obtained above in acetic anhydride (3.9 ml) and dry pyridine (1.7 ml) yielded after the usual work-up the crude acetate (0.2 g) as two spots on tlc. Chromatographic separation on silica gel yielded the major product (0.129 g) in the pure form, which was assigned the structure **14**. An ir showed no absorption in the NH region, and bands at 1775, 1750, 1700, 1633, and 1590 cm^{-1}. The nmr showed signals at δ 7.21 (4 H, q, aromatic), 5.55 (2 H, q, vinylic), 5.22 (1 H, d, carbinolic at C-3), 4.5 (3 H, m, carbinolics C-2 hydromethyl and C-4), 2.03 (3 H, s, acetyl methyl), and 0.88 (3 H, t, terminal methyl). A mass spectrum exhibited M^+ (*m/e* 590), $M^+ - 127$ (*m/e* 463), *m/e* 184 $(-COC_6H_4Br)^+$.

The minor products of acetylation of benzoate from two experiments were pooled and purified by repeated chromatography. The structure **16** for this product follows from the following data. An ir spectrum showed bands at 3400 (NH), 1776, 1750, 1705, 1670, and 1590 cm^{-1}. An nmr had signals at δ 7.61 (4, H, s, aromatic), 6.81 (1 H, s, NH), 5.83 (1 H, d, carbinolic at C-3), 5.50 (2 H, m, vinylic), 4.75 (1 H, m, carbinolic at C-4), 4.61 (2 H, s, carbinolic C-2 hydroxymethyl), 2.08, 1.88 (3 H each, s, acetyl methyl), and 0.88 (3 H, t, terminal $-CH_3$). A mass spectrum exhibited the following peaks: M^+ (*m/e* 650), $M^+ - 60$ (*m/e* 590), $M^+ - 127 + 60$) (*m/e* 463), $M^+ - (184 + 127 + 60)$ (*m/e* 279), and *m/e* 184 $(COC_6H_4Br)^+$.

Mesylation of Diacetate 18.—To a solution of diacetate **18** (0.117 g) in methylene chloride (4 ml) was added triethylamine (0.152 g) followed by methanesulfonyl chloride (0.126 g). The mixture was stirred overnight at room temperature. The reaction mixture was diluted with methylene chloride and washed with sodium bicarbonate, followed by dilute hydrochloric acid and water. The organic liquor was dried and the solvent was removed to yield the crude product (0.155 g). Chromatographic purification gave a pure sample of **21** (0.075 g).

Anal. Calcd for $C_{26}H_{43}NO_9S$ (545): C, 57.0; H, 7.89; N, 2.57; S, 5.86. Found: C, 56.84; H, 7.99; N, 2.59; S, 5.85.

An ir showed bands at 3400 (NH), 1780, 1750, 1690 (carbonyl); nmr δ 6.62 (1 H, s, NH), 5.55 (3 H, m, 2 H vinylic and 1 H C-3 carbinolic), 4.75 (1 H, m, C-4 carbinolic), 4.56 (2 H, d, C-2, CH_2O-), 3.05 (3 H, s, CH_3SO_2), 2.08 (6 H, s, $2CH_3CO-$), 0.89 (3 H, t, terminal $-CH_3$).

Sodium Acetate Treatment of the Mesylate 21.—The mesylate **21** (0.535 g) was dissolved in absolute ethanol (12 ml) in the presence of sodium acetate (0.3 g) and the mixture was refluxed overnight. The reaction mixture was cooled, diluted with ether, washed with water, and dried and the solvent was evaporated. The resulting residue after one crystallization from ether–petroleum ether gave a solid (0.270 g, 73%), mp 87–91°. An analytical sample had mp 94–96°.

Anal. Calcd for $C_{22}H_{35}O_4N$ (377): C, 69.99; H, 9.35; N, 3.71. Found: C, 70.16; H, 9.38; N, 3.64.

An ir showed bands at 3385, 3300 (NH), 1742, 1698, and 1650 cm^{-1} (carbonyl); nmr δ 7.95 (1 H, broad, NH), 7.4 (1 H, d, J = 2 Hz, vinylic), 5.45 (2 H, q, vinylic), 5.02 (1 H, d of t, J = 2 Hz, carbinolic), 2.2 (3 H, s, CH_3CO), 0.9 (3 H, t, terminal $-CH_3$); uv λ_{max}^{EtOH} 246 nm (ϵ 5400).

Treatment of Oxazoline 20 with Sodium Acetate.—To a solution of **20** (30 mg) in absolute ethanol (2.2 ml) was added sodium acetate (60 mg). After refluxing overnight the product was put through a small column of silica gel to yield a product (10 mg) which showed a major spot corresponding to alcohol **19** and a minor spot corresponding to diol **17**. These were separated on a thick layer plate[16] and were shown to be identical with **19** and **17** by comparison of mass spectra with those of the authentic samples.

Registry No.—**1**, 35891-70-4; **1** (tetrahydro tetraacetate), 38223-34-6; **2**, 35891-69-1; **2** (HCl), 38223-36-8; **3**, 38223-37-9; **3** (dihydro), 38223-38-0; **4**, 38223-39-1; **8**, 38223-40-4; **11**, 38223-41-5; **13**, 38223-42-6; **14**, 38223-43-7; **15**, 38223-44-8; **16**, 38337-05-2; **17**, 38223-46-0; **18**, 38223-47-1; **19**, 38223-48-2; **20**, 38223-59-5; **21**, 38223-60-8; **23**, 38223-61-9.

Acknowledgment.—The authors wish to acknowledge the skillful technical assistance of Mr. A. St. Michel and Mrs. P. Toutounji. They also wish to express their appreciation to Dr. G. Schilling and his associates for the analytical data, and to Dr. S. N. Sehgal for the preparation of myriocin on a large scale. The gift of the authentic samples of the 8-ketotetradecanoic and the 11-ketoheptadecanoic acid from Professor Breusch, University of Istanbul, is gratefully acknowledged.

(16) The authors wish to thank Dr. G. Schilling for carrying out this separation and performing the mass spectral comparison.

The Isolation and Structural Elucidation of Eupaserrin and Deacetyleupaserrin, New Antileukemic Sesquiterpene Lactones from *Eupatorium semiserratum*[1,2]

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Received November 14, 1972

Evidence is presented for the assignment of structures for eupaserrin (**1**) and deacetyleupaserrin (**5**), two antileukemic sesquiterpene lactones from *Eupatorium semiserratum* DC. Elemental analysis and high resolution mass spectrometry supported a $C_{22}H_{28}O_7$ molecular formula for eupaserrin (**1**) and a $C_{20}H_{26}O_6$ molecular formula for deacetyleupaserrin (**5**). Acetylation of **5** gave **1** and acetyleupaserrin (**2**), whereas alkaline hydrolysis of **5** gave sarracinic acid. Chemical and spectral evidence indicated the presence of α-methylene-γ-lactone, α,β-unsaturated ester, secondary hydroxyl, and two vinyl methyl groupings in **1** and **5** and suggested that both **1** and **5** were germacranolide dienes. Pyrolysis of **5** gave an oily aldehyde lactone (**6**), and pyrolysis of **2** gave an enol acetate (**3**). Chemical and spectral arguments are advanced for assignment of structure and stereochemistry for **2** and **3** and therefore **1** and **5**.

In the course of a continuing search for tumor inhibitors from plant sources, an alcoholic extract of *Eupatorium semiserratum* DC (Compositae)[3] was found to show significant inhibitory activity *in vivo* against the P-388 leukemia in the mouse and *in vitro* against cells derived from human carcinoma of the nasopharynx

(1) Tumor Inhibitors. LXXXIV. Part LXXXIII: S. M. Kupchan, G. Tsou, and C. W. Sigal, *J. Org. Chem.*, **38**, 1420 (1973).

(2) This investigation was supported in part by grants from the National Cancer Institute (CA-11718) and the American Cancer Society (IC-57), and a contract with the Division of Cancer Treatment, National Cancer Institute (NIH-2099).

(3) Leaves, stems, flowers, and fruits were collected in Florida in Sept 1967. We thank Dr. Robert E. Perdue, Jr., USDA, Beltsville, Md., for supplying the plant material.