(**4b**, R_1 = H; R_2 = Ph) and 3 g (28 mmol) of trimethylsilyl chloride in 8 ml of methylene chloride was held at reflux for 1.5 hr. On distillation there was obtained 3.22 g (91%) of a 95:5 mixture of 2-chloro-2-2-phenylethyl acetate, **2** (R_1 = C_6H_5; R_2 = H), and 2-chloro-1-phenylethyl acetate, **2** (R_1 = H; R_2 = C_6H_5), respectively. This and other compounds prepared by the new procedure are listed in Table I.

It should be pointed out that, while the yields of 1,2-chlorohydrin acetates obtained from 1,3-dioxolanes by method C are comparable to those by method B, the reaction of trimethylsilyl chloride with 2-methoxy-2,5,5-trimethyl-1,3,-dioxane (compound **4** in ref 4) and with 2-methoxy-2-methyl-1,3-dioxepane (compound **5** in ref 4) did not take place to give the expected chloro esters in good yield.

Registry No.—**2a**, 627-68-9; **2b**, 6509-95-1; **2c**, 760-86-1; **2** [R_1 = $(CH_3)_2$; R_2 = H], 6509-93-9; **4a**, 39834-09-8; **4b**, 39904-21-7; **4c**, 42077-65-6; **4** [R_1 = $(CH_3)_2$; R_2 = H], 42077-66-7; trimethylsilyl chloride, 75-77-4.

Structure of the Metabolite LL-S490β from an Unidentified *Aspergillus* Species

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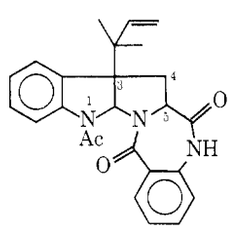
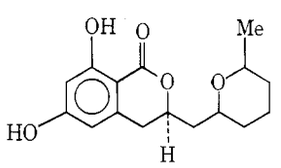
Received June 13, 1973

In relation to a screening program seeking useful biologically active mold metabolites, we examined fermentations of an unidentified *Aspergillus* species. We describe here the structure of a novel benzodiazepinedione (I) designated LL-S490β. In addition to I, we isolated cladosporin[1] (asperentin,[2] II), an antifungal



I



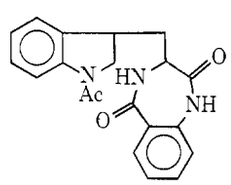
II

metabolite recently obtained from *Cladosporium cladosporiodes*[1] and *Aspergillus flavus*.[2]

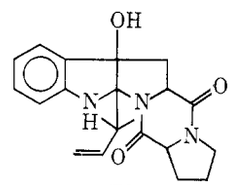
LL-S490β, $C_{25}H_{25}N_3O_3$, mp 238–240°, $[\alpha]_D$ +425° (MeOH), exhibits a mass spectrum characterized by a strong molecular ion at *m/e* 415 in addition to significant peaks at *m/e* 373 (M − 42), 346 (M − 69), and the base peak at 304 [M − (42 + 69)]. A high-intensity peak at *m/e* 130 is assigned to the indoline-3-methylene ion.[3] The general appearance of the uv spectrum [λ_{max} 210 (ε 61,000), 245 (22,000), and 284 nm (sh, 3940)] is very reminiscent of the *N*-acylindoline chromophore,[4] although the high extinction values indicate the presence of an additional chromophoric unit.

The nmr spectrum of I discloses the presence of eight aromatic proton signals between δ 6.83 and 8.17. A sharp 3-H singlet at δ 2.60 is assigned to the methyl of an *N*-acetyl group. Two tertiary *C*-methyl signals resonate at δ 1.02 and 1.21 and, in conjunction with the loss of 69 mass units in the mass spectrum, are assigned to the inverted γ,γ-dimethylallyl group. Consistent with this is the appearance of the very characteristic ABX pattern of the three vinyl protons of the C_5 moiety between δ 5.16 and 5.92.[5]

The ir spectrum of I shows absorption at 3300, 1689, and 1647 cm^{-1} assigned to NH and amide functionalities, respectively. In fact the latter two absorptions strongly suggest the presence of a dipeptide system.[6] Consideration of the molecular formula and the functionality described above suggests the combination of a tryptophane portion with anthranilic acid to give the partial structure III below. The uv of 3,4-dihydro-



III



IV

4-methyl-1*H*-1,4-benzodiazepine-2,5-dione [λ_{max} 215 nm (ε 32,100) and 291 (2180)][7] superimposed with that of the *N*-acylindoline system accounts for the observed uv spectrum of I.

The final molecular assembly was arrived at by examination of an ABX pattern in the nmr spectrum between δ 2.46 and 3.90 assigned to the geminal protons at C-4 and the methine hydrogen at C-5. The geminal pair resonate as four-line patterns at δ 2.46 and 3.42 with J_{AB} = 14, J_{AX} = 8.5, and J_{BX} = 8.0 Hz. The H-5 signal appears at δ 3.90 as an apparent triplet (J = 8 Hz). The similar J values of the corresponding nmr system in brevianamide E[5] (IV) provide a good analogy.

Placement of the inverted terpene unit at C-3 is dictated by the fact that the aforementioned geminal hydrogens are spin coupled only to the C-5 methine hydrogen. A sharp 1-H singlet at δ 6.00 is assigned to the C-2 methine hydrogen on comparison with the spectrum of a model compound.[4b] The singlet nature of this signal supports the absence of a proton at C-3.

An exchangeable 1-H singlet (br d) at δ 8.5 is attributable to the NH of the benzodiazepinedione ring.

The occurrence of I with the inverted C_5 unit at C-3 is unusual and raises the question as to the mode of incorporation of the terpene moiety into the indolyl system. A chemical precedent comes from the work of Bycroft and Landon,[8] who incorporated the inverted C_5 grouping at C-3 by a thio-Claisen rearrange-

(1) P. M. Scott, W. van Walbeek, and W. M. MacLean, *J. Antibiot.*, **24**, 747 (1971).

(2) J. F. Grove, *J. Chem. Soc., Perkin Trans. 1*, 2400 (1972).

(3) J. H. Benyon, R. A. Sanders, and A. E. Williams, "The Mass Spectra of Organic Molecules," Elsevier, New York, N. Y., 1968, p 303.

(4) (a) A. I. Scott, "Ultraviolet Spectra of Natural Products," Pergamon Press, Oxford, 1964, p 298; (b) M. Ohno, T. F. Spande, and B. Witkop, *J. Amer. Chem. Soc.*, **92**, 343 (1970).

(5) A. J. Birch and J. J. Wright, *Tetrahedron*, **26**, 2329 (1970).

(6) K. Blaha, J. Smolikova, and A. Vitek, *Collect. Czech. Chem. Commun.*, **31**, 4296 (1966).

(7) P. K. Martin, H. Rapoport, H. W. Smith, and J. L. Wong, *J. Org. Chem.*, **34**, 1359 (1969).

(8) B. W. Bycroft and W. Landon, *Chem. Commun.*, 967 (1970).

ment of a dimethylallyl 2-indolyl sulfonium salt. It is of interest that a number of related metabolites, such as euchinulin,[9] the brevinamides,[5] and austamide,[10] contain the inverted C_5 unit at C-2.[11]

Experimental Section

The melting points were determined on a Fisher-Johns melting point block. Nmr spectra were recorded with a Varian A-60D in $CDCl_3$; shifts are expressed in δ values (parts per million) from tetramethylsilane as internal standard, and coupling constants are expressed in cycles per second (hertz). In nmr descriptions, s = singlet, d = doublet, t = triplet, m = multiplet, and q = quartet. Infrared spectra were taken on a Perkin-Elmer Model 137 Infracord and ultraviolet spectra on a Cary Model 11.

Isolation of I.—The whole mash from a 30-l. fermentation was extracted with an equal volume of ethyl acetate at pH 5.0. The extract was concentrated to dryness and the residue was partitioned between methanol and heptane to remove fatty material. Evaporation to dryness of the methanol portion gave ~25 g of a crude residue. Twelve grams of this residue was chromatographed over a 500-g silica gel column (acid washed) packed in methylene chloride. A gradient elution between 0.5% methanol–methylene chloride and 3% methanol–methylene chloride provided cladosporin (II, 110 mg) after evaporation of the solvent and crystallization from ethyl acetate–benzene, mp 186–187°. Further elution gave the benzodiazepinedione I (185 mg) after removal of the solvent and crystallization from ethyl acetate–benzene: mp 238–240°; $[\alpha]_D$ +425° (*c* 0.20, MeOH); ir (KBr) 3300, 1689, and 1647 cm^{-1}; λ_{max}^{MeOH} 210 nm (ϵ 61,000), 245 (22,000), and 284 (sh, 3940); nmr ($CDCl_3$) δ 1.02 and 1.21 (3 H, s), 2.60 (3 H, s), 2.46 (q, J_{AB} = 14, J_{AX} = 8.0 Hz), 3.42 (q, J_{AB} = 14, J_{BX} = 8.5 Hz), 3.90 (t, J = 8 Hz), 5.16 (m, AB of vinylidene), 5.92 (q, J_{trans} = 18, J_{cis} = 9.5 Hz, X of vinylidene), 6.00 (1 H, s), 6.83–8.17 (8 H, m), and 8.45 (1 H, s); mass spectrum *m/e* 415.18919 (calcd for $C_{25}H_{25}N_3O_3$, 415.18959).

Chromatography of the remaining portion of the crude ethyl acetate concentrate gave a total of 225 mg of II and 389 mg of I.

Acknowledgments.—We wish to thank Dr. H. Tresner and Miss Jean Hayes for culture isolation and identification, Mr. A. Shay for large-scale fermentations, Mr. M. Dann for large-scale work-ups, Mr. W. Fulmor and Mr. G. Morton for the uv and nmr spectra, and Dr. G. Van Lear for the mass spectrum [direct inlet MS 9 (AEI)].

Registry No.—I, 42230-55-7; II, 35818-31-6.

(9) A. Quilico, *Res. Progr. Org.-Biol. Med. Chem.*, **1**, 1964 (1964); for a recent study on the biosynthesis of echinulin, see C. M. Allen, Jr., *Biochemistry*, **11**, 2154 (1972).

(10) P. S. Steyn, *Tetrahedron Lett.*, 3331 (1971).

(11) A proposal for the echinulin-type metabolites has been put forth by Cosnati and Pochini [*Chem. Commun.*, 1328 (1970)]. On the basis of model reactions, they point out the feasibility of a primary attack at N-1 (*e.g.*, lanosulin[12]) followed by rearrangement to introduce the inverted γ,γ-dimethylallyl group at C-2. This postulate does not appear to be relevant to the introduction of the C_5 moiety at C-3.

(12) D. T. Dix, J. Martin, and C. E. Moppett, *Chem. Commun.*, 1168 (1972).

Cyclization of Azidoformates

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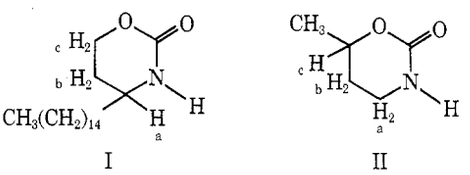
Received June 25, 1973

The major product of the thermolysis of *n*-octadecyl azidoformate in cyclohexane is *n*-octadecyl *N*-cyclohexylcarbamate. In addition, two isomeric minor products, with the empirical formula $C_{19}H_{37}O_2N$, are found. One, obtained in 5% yield, is the five-membered ring compound formed by "backbiting" of the nitrene, 4-*n*-hexadecyloxazolidin-2-one, as shown by comparison of its infrared and nmr spectra with those of an authentic sample of the 4-ethyl derivative.[2,3]

Although common sense dictated that the other isomer, obtained in 8% yield, should be the corresponding six-membered ring compound, 4-*n*-pentadecyltetrahydro-2*H*-1,3-oxazin-2-one (I), the nmr spectrum in comparison with an "authentic" sample of the corresponding 4-methyl derivative seemed to eliminate this possibility; the difficulty arose because in the "4-methyl derivative" spectrum the two protons adjacent to O are upfield from the one adjacent to N, whereas in the octadecyl compound the reverse is true.[2]

Edwards[4] suggested that the large alkyl group in I imparts conformational rigidity to the ring and causes the signal of one of the hydrogens in the 6 position to overlap that of the hydrogen in the 4 position, adjacent to the N atom. We did not consider this a very likely explanation and decided to reinvestigate the problem.[5]

There is now no doubt that the unknown is indeed the six-membered ring isomer (I), whereas the "authentic 4-methyl derivative" is 6-methyltetrahydro-2*H*-1,3-oxazin-2-one (II). Table I summarizes the nmr chemical shifts observed for the various proton groups in the two compounds. Time averaging in the presence of $Eu(dpm)_3$ showed H_a in I to be a quintet, consistent with the assigned structure. The fact that the H_c protons occur as a narrow (*ca.* 13 Hz) multiplet is consistent with a six-membered ring, as is the absence of an amide II band in the infrared spectrum.[2] The fact that the single proton (H_a) occurs at higher field than the two



I II

TABLE I

NMR SPECTRA AND PEAK ASSIGNMENTS FOR ISOMERIC ALKYL-2*H*-1,3-OXAZIN-2-ONES

Proton group	δ, ppm from TMS (rel area) I	II
H_a	3.4 (1)	3.36 (2.1)
H_b	1.9 (1.8)	1.9 (2)
H_c	4.23 (2)	4.41 (1.0)
NH	6.45 (0.8)	7.1

(1) Hercules Research Center Contribution No. 1617.

(2) D. S. Breslow, T. J. Prosser, A. F. Marcantonio, and C. A. Genge, *J. Amer. Chem. Soc.*, **89**, 2384 (1967).

(3) Our recent nmr studies have shown that the nmr chemical shift assignments given in ref 1 for 4-*n*-hexadecyloxazolidin-2-one and the corresponding ethyl derivative, which were based on poorly resolved spectra obtained on a primitive instrument, were in error. Spectra run on a modern high-resolution instrument, confirmed by proton decoupling and the use of lanthanide shift reagents, show that the ring methylene protons adjacent to oxygen are nonequivalent and occur at δ 4.0 and 4.6. The ring methyne proton adjacent to nitrogen is observed at δ 3.8.

(4) O. E. Edwards, "Nitrenes," W. Lwowski, Ed., Interscience, New York, N. Y., 1970, p 236.

(5) We are indebted to Dr. C. A. Genge and Mrs. E. I. Edwards of the Hercules Research Center for the preparation and isolation of a fresh sample of isomer.