Circular Dichroism Studies on the Azoxy Chromophore of the Antibiotics LL-BH872α and Elaiomycin

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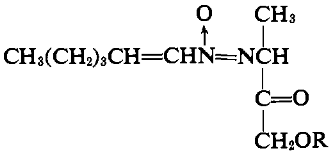
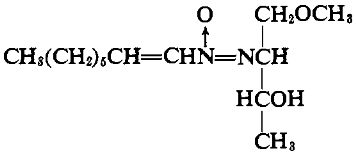
Abstract: Antibiotics LL-BH872α and elaiomycin each display Cotton effects due to the asymmetric perturbation of an azoxy chromophore by a vicinal asymmetric center of the D configuration. The intensities of the Cotton effects of LL-BH872α are much greater than those for elaiomycin and may be attributed to the coupling through space of the azoxy system with a homoconjugated carbonyl electronic system. From the interpretation of circular dichroism studies and in conjunction with stability data, the complete characterizations of LL-BH872α and elaiomycin are proposed as (3*R*)-1-hydroxy-3-(1′-*cis*-hexenyl-*trans*-azoxy)-2-butanone and (2*S*,3*S*)-4-methoxy-3-(1′-*cis*-octenyl-*cis*-azoxy)-2-butanol, respectively. The double-humped circular dichroism curves of both antibiotics are attributed to equilibria of free and asymmetrically solvated species in the solvents used.

In a previous report[1] the unstable liquid antifungal agent LL-BH872α has been characterized as D-1-hydroxy-3-(1′-*cis*-hexenylazoxy)-2-butanone (Ia). This antibiotic and the previously characterized[2] liquid tuberculostatic agent elaiomycin (II) both contain an asymmetric carbon atom of the D configuration adjacent to an α,β-unsaturated azoxy chromophore. Since α,β-unsaturated azoxy compounds have seldom been encountered[3] as far as we are aware, neither optical rotatory dispersion (ORD) nor circular dichroism (CD) studies have been carried out on this chromophore. Since both antibiotics were available to us[4] we undertook CD studies with rewarding results. The classification by Moscowitz[5] of chromophores into two limiting types, namely symmetrical chromophores such as the carbonyl group which are asymmetrically perturbed and inherently dissymmetric chromophores, is widely accepted. Cotton effects associated with the former type usually have molecular ellipticities of the order 10^2 to 10^3 while the latter kind, frequently consisting of twisted conjugated or homoconjugated carbonyl and olefinic electronic systems, display Cotton effects with ellipticities of 10^4 or greater. Our studies on the azoxy chromophore of Ib show that while this is an asymmetrically perturbed symmetric chromophore, it does in fact exhibit some of the characteristics of an inherently dissymmetric chromophore.



$$\mathrm{CH_3(CH_2)_3CH{=}CH\overset{\overset{O}{\uparrow}}{N}{=}N\overset{\overset{CH_3}{|}}{C}H{-}C({=}O){-}CH_2OR}$$

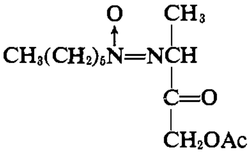
Ia, R = H
b, R = Ac



$$\mathrm{CH_3(CH_2)_5CH{=}CH\overset{\overset{O}{\uparrow}}{N}{=}N\overset{\overset{CH_2OCH_3}{|}}{C}H{-}HCOH{-}CH_3}$$

II

Because of the instability of Ia our studies were carried out on the more stable acetate derivative Ib. The CD curve of Ib in trifluoroethanol has a strong negative Cotton effect at 230 mμ and an equally strong positive Cotton effect at 259 mμ (Figure 1). The magnitude of the molecular ellipicities $\Theta = -1.6 \times 10^4$ and 1.9×10^4, respectively, is such that the presence of an inherently dissymmetric chromophore might be suspected. Clearly the α,β-unsaturated electronic system is not involved since the reduced derivative of Ib, compound III, shows two Cotton effects of approximately similar rotatory strengths located at 216 and 245 mμ, respectively (Figure 2). The blue shift of the two effects on elimination of conjugation is an indication that we are dealing with a π → π* transition.[6] The approximate correspondence of the lower Cotton effect at 230 mμ of compound Ia with its strong uv absorption maximum at 237 mμ (9000) further bolsters this assignment.[7]



$$\mathrm{CH_3(CH_2)_5\overset{\overset{O}{\uparrow}}{N}{=}N\overset{\overset{CH_3}{|}}{C}H{-}C({=}O){-}CH_2OAc}$$

III

The CD curve of compound II (Figure 3) exhibits a weak positive Cotton effect at 232 mμ ($\Theta = 1.25 \times 10^3$) and an even weaker negative effect at 259 mμ ($\Theta = -3.3 \times 10^2$). The order of these molecular ellipticities is that normally associated with a symmetric chromophore. Since compound II does not contain a carbonyl group, it was reasoned that the strong Cotton effects of compound Ib arise because of coupling through space[8] of the homoconjugated carbonyl electronic system with the azoxy chromophore. To test this idea, elaiomycin in ether solution was oxidized, using an acidic aqueous solution of potassium dichromate, to

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[1] W. J. McGahren and M. P. Kunstmann, *J. Amer. Chem. Soc.*, **91**, 2808 (1969).

[2] C. L. Stevens, B. T. Gillis, and T. H. Haskell, *ibid.*, **81**, 1435 (1959).

[3] B. T. Gillis and J. D. Hagarty, *J. Org. Chem.*, **32**, 95 (1967).

[4] We wish to thank Dr. T. H. Haskell of the Parke Davis Co. who graciously supplied us with samples of elaiomycin.

[5] A. Moscowitz in "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," G. Snatzke, Ed., Heyden, London, 1967, Chapter 3.

[6] J. G. Calvert and J. N. Pitts, Jr., "Photochemistry," John Wiley & Sons, Inc., New York, N. Y., 1966, p 261.

[7] G. McConnell, *J. Chem. Phys.*, **20**, 700 (1952).

[8] K. Mislow in "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," G. Snatzke, Ed., Heyden, London, 1967, Chapter 10.

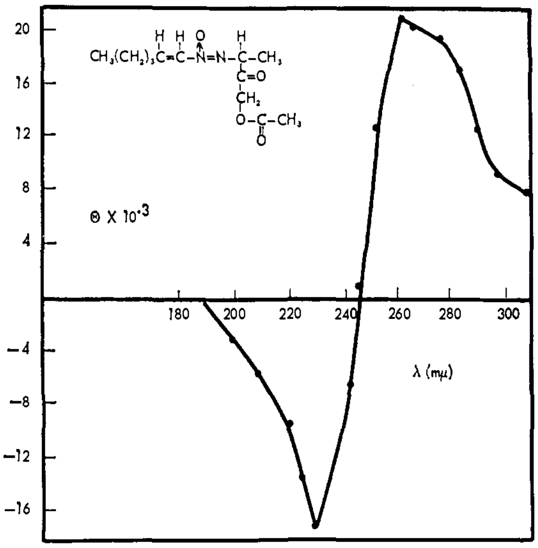


Figure 1. CD curve of LL-BH872α acetate Ib in trifluoroethanol.

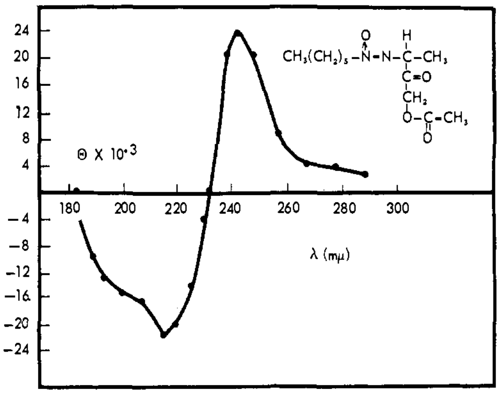


Figure 2. CD curve of reduced LL-BH872α acetate III in trifluoroethanol.

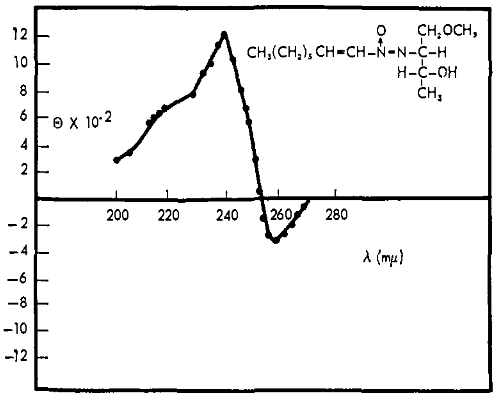


Figure 3. CD curve of elaiomycin II in trifluoroethanol.

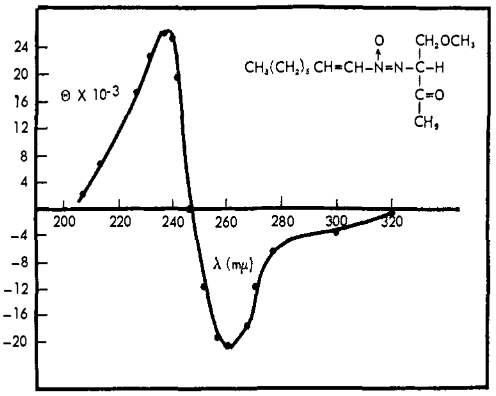
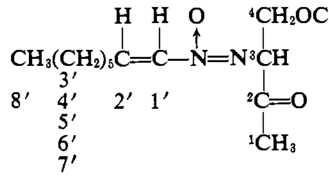


Figure 4. CD curve of oxidized elaiomycin IV in trifluoroethanol.

compound IV, the structure of which was verified using spectral data. The CD curve of IV (Figure 4) shows a



$$\begin{array}{l} \quad\quad\quad\quad\;\; \mathrm{H}\;\;\mathrm{H}\;\;\mathrm{O}\quad\;\; {}^{4}\mathrm{CH_2OCH_3} \\ \mathrm{CH_3(CH_2)_5C{=}C{-}\overset{\uparrow}{N}{=}N^3CH} \\ \quad\;\; 3' \\ 8'\;\; 4'\quad 2'\;\; 1' \quad\quad {}^{2}\mathrm{C{=}O} \\ \quad\;\; 5' \\ \quad\;\; 6' \quad\quad\quad\quad\;\; {}^{1}\mathrm{CH_3} \\ \quad\;\; 7' \end{array}$$

IV

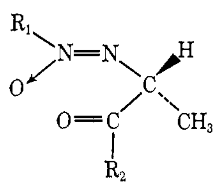
strong positive Cotton effect at 230 mμ (Θ = 2.4 × 10^4) and a strong negative effect at 260 mμ (Θ = −2.1 × 10^4). Clearly the enhancement of the molecular ellipticities in going from II to IV to the magnitude observed for those of Ib is due to orbital mixing of the newly formed, homoconjugated carbonyl system with the azoxy electronic system. Despite the enhancement of ellipticities, the chiroptic behavior of compound IV is basically still due to asymmetric perturbation of the symmetric azoxy chromophore by a vicinal asymmetric center. It is interesting to note in passing that while the molecular ellipticities are greatly increased in going from compound II to compound IV there is no corresponding intensification of uv absorption. Compound II has a uv maximum at 238 mμ (ε 11,000) while IV peaks at 237 mμ (ε 9000). This is almost the opposite of the effects observed by Kessar, *et al.*,[9] in their work with the coupled chromophores of steroidal γ-nitro ketones. They observed a 20-fold increase in the uv absorption of a γ-nitro ketone steroid due to coupling between nitro and carbonyl electronic systems while the circular dichroism curve of the same compound displayed normal carbonyl molecular ellipticity.

Two features of the CD curve of compounds Ib and II remain to be explained, namely, the reversal of the signs of the Cotton effects in II as opposed to those of Ib and second, the presence of two Cotton effects in the curve of each.

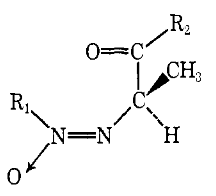
Compounds Ib and II both have the same chromophore vicinally perturbed by centers of the same configuration. Hence, we would normally expect Cotton effects of the same sign. It must be admitted, however, that even in a given solvent, a few cases are recorded of homologous compounds of identical chirality exhibiting Cotton effects of opposite sign.[10] Nevertheless, we feel that the most likely explanation of the reversal of the signs of the Cotton effects in compound Ib as opposed to those of II and IV is due to different configurations of the azoxy group in these compounds. Construction of Dreiding models of *cis*- and *trans*-azoxy chromophores with adjacent asymmetric centers of the same configuration as indicated below is useful to illustrate the situation.

[9] S. V. Kessar, M. Singh, and Y. P. Gupta, *Tetrahedron Letters*, 583 (1969).

[10] E. Bach, A. Kjaer, R. Dahlbom, T. Walle, B. Sjoberg, E. Bunnenberg, C. Djerassi, and R. Records, *Acta Chem. Scand.*, 20, 2781 (1966).

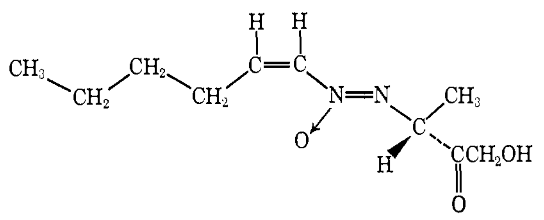


trans-azoxy model

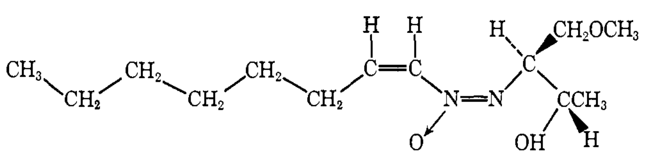


cis-azoxy model

In the models, if the *cis*- and *trans*-azoxy chromophores and any one of the three substituents of the asymmetric center are aligned in the plane of symmetry of the chromophore then the remaining two substituents of the asymmetric center are reversed relative to this plane. Consequently, one would expect opposite Cotton effects from the two azoxy configurations when the vicinal centers of asymmetry are of the same configuration. This observation is analogous to the octant rule statement that the sign of the contribution which a given perturbing atom at point $P(x, y, z)$ makes to anomalous rotatory dispersion ($n \rightarrow \pi^*$ transition of the carbonyl) will vary as the sign of the product (xyz) of its coordinates.[11] The assignment of a specific configuration of the azoxy group to either of the antibiotics cannot be made on the basis of CD data alone. Brough and Littgoe[12] in their studies on the *cis* and *trans* isomers of 4-chlorobenzylazoxybenzene have shown that the *cis* isomer is by far the more stable of the two isomers. Since compounds II and IV are stable oils these materials probably have the *cis* configuration of the azoxy group. Compound I is very unstable and hence, probably has the *trans* configuration of the same group. It must be admitted though that part of the instability of I is due to the presence of the hydroxymethyl group adjacent to the carbonyl since when the isomerization potential of the combination is eliminated by protecting the hydroxy group as the acetate, the low-temperature stability is improved. However, the acetate Ib still rearranges under very mild conditions and this instability may be attributed to the *trans*-azoxy configuration.



V



VI

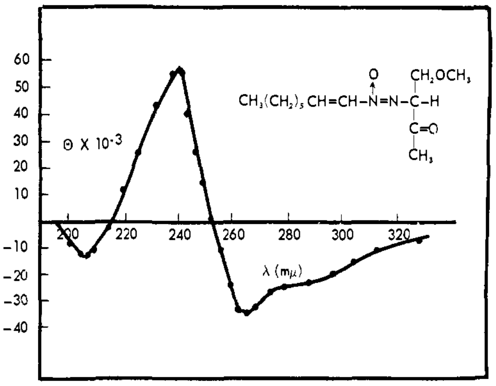


Figure 5. CD curve of oxidized elaiomycin IV in cyclohexane.

On the basis of this evidence, we propose the complete structural characterizations of the antibiotics LL-BH872α and elaiomycin as (3*R*)-1-hydroxy-3-(1′-*cis*-hexenyl-*trans*-azoxy)-2-butanone (V) and (2*S*,3*S*)-4-methoxy-3-(1′-*cis*-octenyl-*cis*-azoxy)-2-butanol (VI), respectively. There remains the explanation of the two Cotton effects in the CD curves of the compounds Ib, II, III, and IV each of which contain a single effective chromophore. Wellman, *et al.*,[13] have discussed the presence of multiple Cotton effects arising from the $n \rightarrow \pi^*$ transition of a single carbonyl chromophore. They showed that two oppositely signed Cotton effects separated by about 30 mμ will arise whenever two Cotton effects of similar amplitudes but opposite signs are superimposed with their individual maxima separated by from 1 to 20 mμ. The double-humped curves arise because of conformational and/or solvational equilibria. In the case of a rigid molecule such as isofenchone, Moscowitz, *et al.*,[14] attributed the presence of two Cotton effects to solvational effects since a conformational equilibrium is highly unlikely. Conformational equilibria cannot be ruled out for the nonrigid structures I–IV. In the case of compounds Ib and IV one would expect the same sequence of groups around the asymmetric centers based on steric weighing of the substituents, and hence, the probability of similar predominant conformers for these materials. However, we believe that the double-humped CD curves of compounds I through IV are probably due to asymmetric solvation. Proof of a solvational complex equilibrium is usually obtained by low-temperature CD studies where the lack of thermal energy to disrupt the complex gives rise to enhanced Cotton effects due to solvated species coupled with decreased effects due to the free material. The apparatus for low-temperature CD work was not available to us, but we did examine the CD curve of compound IV in cyclohexane (Figure 5) in which solvent we expected less solvation to occur and consequently an enhancement of the Cotton effect due to the free species. Our expectation was borne out as the ratio of free species to asymmetrically solvated complex goes from 1.1 in trifluoroethanol to 1.7 in cyclohexane.

The Cotton effect due to the free species should correspond roughly with the uv absorption maximum of the material. Hence, the Cotton effect at 230 mμ (Figure 4) should correspond to free compound IV while the Cotton effect at 260 mμ should correspond to the asymmetrically solvated complex. This red shift on solvation may be considered one further piece of evidence pointing to a $\pi \rightarrow \pi^*$ transition.[6] Also there is no

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[11] G. Wagniere, *J. Amer. Chem. Soc.*, **88**, 3937 (1966).

[12] J. N. Brough, B. Littgoe, and (in part) P. Waterhouse, *J. Chem. Soc.*, 4069 (1954).

[13] K. M. Wellman, P. H. A. Laur, W. S. Briggs, A. Moscowitz, and C. Djerassi, *J. Amer. Chem. Soc.*, **87**, 66 (1965).

[14] A. Moscowitz, K. M. Wellman, and C. Djerassi, *Proc. Nat. Acad. Sci., U. S.*, **50**, 799 (1963).

obvious difference in fine structure in the two curves. Such fine structure differences are usually noted with changing solvent polarity when the transition involved is $n \rightarrow \pi^*$.[15]

The locations of the Cotton effects of compound IV at 237 mμ ($\Theta = 5.7 \times 10^4$) and 267 mμ ($\Theta = -3.3 \times 10^4$) in cyclohexane are shifted to higher wavelengths from those observed in the more polar trifluoroethanol. Such a shift is normally strong evidence for the assignment of an $n \rightarrow \pi^*$ transition. However, Sidman[16] points out that $\pi \rightarrow \pi^*$ transitions which shift to shorter wavelengths in polar solvents are known in heteropolar systems such as pyridine N-oxide. In view of this and because of the other pieces of evidence already cited, we feel that we are dealing with a $\pi \rightarrow \pi^*$ transition.

In addition to the Cotton effects already discussed, compound IV in cyclohexane exhibits an additional Cotton effect at 207 mμ ($\Theta = -1.2 \times 10^4$). We have no ready explanation of this effect other than that the transition involved could be olefinic in nature as suggested by Sandman and Mislow,[17] who observed a similar Cotton effect at 207.5 mμ in their CD studies on (+)-2-methylenebenznorbornene.

Experimental Section

The CD studies were carried out at room temperature using a Cary 60 spectropolarimeter with CD attachment. The compound LL-BH872α acetate Ib was examined in trifluoroethanol at a concentration of 1.18 mg/ml using a 0.2-mm cell. Compound III, reduced LL-BH872α acetate, was studied at a concentration of 2.10 mg/ml in trifluoroethanol in a 0.2-mm cell while the CD curve of elaimocyin was made on a 2.60 mg/ml solution in trifluoroethanol in a 0.2-mm cell.

Oxidation of Elaiomycin. Approximately 54 mg (just over 0.2 mmole) of elaiomycin in 4–5 ml of ether was stirred with a solution consisting of 50 mg of potassium dichromate and 0.1 ml of concentrated sulfuric acid in 1.0 ml of water. After 1.5 hr stirring, thin-layer chromatography (tlc) on silica gel using the developing system hexane–ethyl acetate (60:40) showed that about 20% reaction had occurred. The reaction mixture was stored overnight in the refrigerator. Stirring at room temperature was continued the following day for 3.5 hr during which time the ether volume was replenished several times and two lots of 20 mg of potassium dichromate were added. Tlc then showed about 80% reaction had occurred so the ether phase was recovered and concentrated to 60 mg of faintly yellow oil which was chromatographed over 20 g of acid-washed silica gel (Davison grade 62) using the solvent system hexane–ethyl acetate (95:5). The desired product was eluted in the fourth and fifth holdback volumes. Evaporation of the solvent yielded 30 mg of colorless oil. The molecular ion by mass spectrum is *m/e* 256. The ir spectrum has a sharp carbonyl absorption band at 1715 cm^{-1} while the 3100–4000-cm^{-1} area is free of absorption bands. The nmr spectrum in carbon tetrachloride is definitive for the structure 4-methoxy-3-(1′-*cis*-octenylazoxy)-2-butanone. The 8′ terminal methyl signal is at δ 0.87. The methylene protons of the 4′, 5′, 6′, and 7′ carbon atoms appear as a broad singlet at 1.30. The sharp singlet at 2.12 integrating for three protons belongs to the ketonic methyl group of C_1. The multiplet at 2.60 which accounts for two protons is due to the allylic methylene group of C_3'. The methoxy substituent of carbon 4 appears at 3.32. The doublet ($J = 6$ Hz) at 4.45 is attributable to the single proton of carbon 3. The vinyl proton of C_1' appears as a doublet of triplets at 6.77 ($J =$ 9–10 Hz indicative of *cis* coupling) while the remaining vinyl proton of C_2' is a multiplet at 5.70. The uv of oxidized elaiomycin has λ_{max} at 238 mμ (ϵ 9000) in methanol; $[\alpha]^{25}_D -87.8° \pm 2.0$ (*c* 0.148, methanol). The CD curves of oxidized elaiomycin IV were made on a 1.04 mg/ml solution in trifluoroethanol and on a 0.97 mg/ml solution in cyclohexane. The cell width in each case was 0.2 mm.

Acknowledgments. We wish to thank Professor Murray Goodman of the Polytechnic Institute of Brooklyn for the CD curves and our colleagues, Mr. W. Fulmor and his associates, for the spectral and rotational data.

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[15] W. D. Closson and P. Haug, *J. Amer. Chem. Soc.*, **86**, 2384 (1963).

[16] J. W. Sidman, *Chem. Rev.*, 689 (1958).

[17] D. J. Sandman and K. Mislow, *J. Amer. Chem. Soc.*, **91**, 645 (1969).

Chemical Ionization Mass Spectrometry of Complex Molecules. II. Alkaloids

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Abstract: The chemical ionization mass spectra of 29 alkaloids, representing nine of the eighteen major alkaloid families, have been measured using methane as reactant gas. These spectra are discussed in terms of the structure of the compounds, with particular reference to their conventional electron impact mass spectra. The quasi-molecular ion $(M + 1)^+$ is invariably more relatively abundant in the chemical ionization mode than is the molecular ion in the electron impact mode. Both spectra supply structural data that is often of a complementary nature, *via* fragmentation.

No area of organic chemistry has remained unaffected by mass spectrometry, which during the last decade has been developed into a routine analytical technique of uniquely high sensitivity, having particular value in the general field of structure determination. The high sensitivity of the method has led to its rapid acceptance and exploitation by those working on the characterization of natural products. In this area alkaloids have been the subject of a very large number of reports.[1]