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CHIROPTICAL PROPERTIES OF STREPTONIGRIN AND A COMMENT ON ATROPISOMERISM IN HETEROCYCLIC COMPOUNDS

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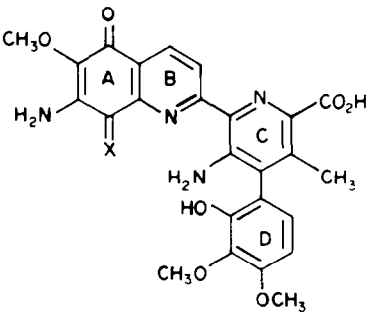
Abstract—Chiroptical properties of streptonigrin (from *Streptomyces flocculus*) and its monoxime are recorded. This is the first case of atropisomerism arising from a phenylpyridine in a natural product, and from any 4-phenylpyridine.

Christie and Kenner resolved[1] 6-6'-dinitrodiphenic acid and discovered enantiomerism in biphenyls. The phenomenon (atropisomerism) is now well known, but its consequences are not always appreciated. Several skewed biphenyls occur in nature. Hexahydroxydiphenic acid, found bound as a glycoside in tannin extracts from Myrobalans had[2] $[\alpha]_D^{20} = -25.5 \pm 1.5°$ (*c* 1.05, EtOH) and $-127 \pm 2°$ (*c* 1.25, 0.1 M NaOH).

However, among the examples[3] of naturally occurring atropisomerism there has been no report of a skewed bipyridine or phenyl pyridine. We describe here the chiroptical properties of streptonigrin and comment on some related atropoisomeric contributions to rotations.

Streptonigrin, (produced by *Streptomyces flocculus*), an antibiotic active against a broad spectrum of bacteria and which inhibits various tumours was shown by the detailed chemical studies[4] of Woodward and his colleagues to have structure I:(X = O).



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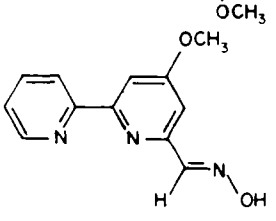
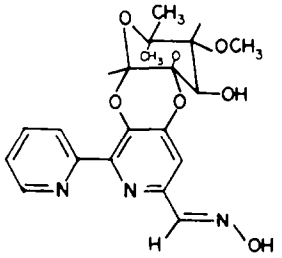


11A



11D

The crystal structure of streptonigrin (as its solvate with ethyl acetate) has recently been solved[5] by Chiu and Lipscomb. With labelling as in I, the rings A, B, and C are essentially co-planar, and make an angle of 85° with the crystallographic c-axis, while ring D (which is out of that plane) makes an angle of 162° with the c-axis. The space group is the enantiomorphous $P2_12_12_1$, showing that each individual crystal is optically active, containing only one hand of the skewed streptonigrin molecule. Such enantiomorphism could arise through spontaneous resolution of the racemic solution, or by selected handed crystallisation of a rapidly equilibrating "racemic" mixture or, a very common case among natural products, because the molecules in solution are themselves all of one hand.

Streptonigrin is in fact optically active in solution. We report here the CD, measured in solution. There have been no previous measurements of chiroptical properties. These properties offer a novel method of studying detailed interactions between streptonigrin and such molecules as nucleic acids.

The intensities of the CD bands run parallel with the extinction coefficients of the electronic absorption spectra. This phenomenon (Fig. 1) is characteristic[6] of molecules which possess "inherently dissymmetric" chromophores. The shorter wavelength negative Cotton effect corresponds to the biphenyl conjugated band, which lies at 245 nm. This sign suggests that streptonigrin has the "S" configuration.

Streptonigrin has the 1,2-di-imine fragment found in

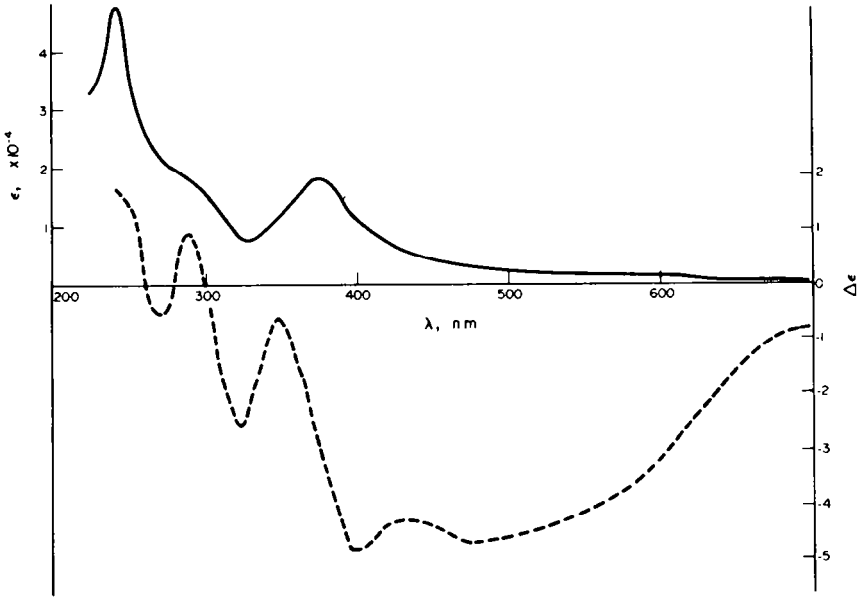


Fig. 1. Isotropic absorption (———) and circular dichroism (- - - - -) spectra for solutions of streptonigrin in ethanol.

caerulomycin and in pyrimine, and might therefore be expected to show metal-binding properties. In fact, when Zn(II) or Fe(II) is added to streptonigrin, the shoulder at 500 nm in the CD spectrum disappears. Since this band arises from the quinone group,[7] these metals bind to streptonigrin via the quinone group.[8]

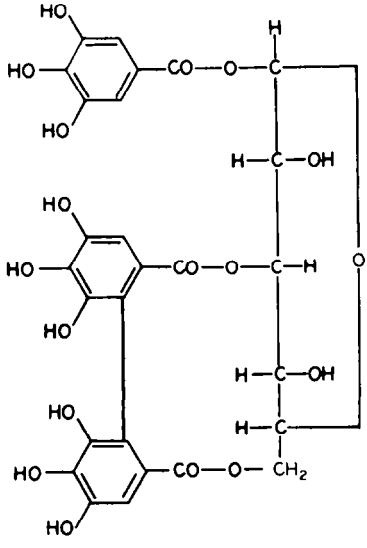
Accepting that the solid-state conformation is retained in solution in ethyl acetate, the optical activity arises from the skewed rings C and D. Rotation about the bond between rings C and D is prevented by steric hindrance of amine and methyl groups of one ring and the OH group of the other. The rate of racemization of the compound would be slow because of the buttressing effect,[18] caused by bulky substituents in the 3 and 3' positions.

A solution originally containing streptonigrin was found to be optically active even after the solute had been allowed to decompose. The shoulder at 550 nm, due essentially to the aminoquinone chromophore, disappears, while the band at 370 nm is shifted towards shorter wavelengths. Therefore the compound must decompose via the aminoquinone group: the optical activity stemming from the skewing of rings C and D remains intact. This observation comments on the chiral stability of rings C and D as does the following. Streptonigrin monoxime,[9] which is much less reactive than the parent streptonigrin, is made by boiling streptonigrin with hydroxylamine in pyridine for 3 hr. The product is still strongly optically active, although the band at 500 nm, due essentially to the quinone moiety, disappears. Clearly, racemization is slow.

The optical activity of the monoxime is much more evident in the presence of iron(II) sulphate (Fig. 2). This is because a complex species forms, in which the helicity of the skewed bi-aryl is transmitted to the electronic transitions localized (in part) on the metal orbitals. The structure of the monoxime given[9] (naming AB of streptonigrin as a quinoline derivative) is[10] a 8-oxime (I, X = NOH) rather than the alternative 5 position (which seems less hindered). However, the spectra do indeed suggest such a structure. The stoichiometry of Fe(II): streptonigrin monoxime was found to be 1 : 2 by Job's method of continuous variation using CD.

A space-filling model of streptonigrin monoxime shows that rings B and C must be disposed *trans*. The most likely binding sites are the oxime N and the amino N of rings A and C, i.e. streptonigrin monoxime possibly acts as a terdentate ligand.

Among other naturally occurring 2,2'-bipyridyls are the caerulomycins (isolated from *Streptomyces caeruleus*). Caerulomycin A (IIA) seems unlikely to manifest stable atropisomerism. However, a recent discussion[11] of the absolute configuration at C(2) in the sugar of caerulomycin D(IID) was based on arguments using values of



(III)

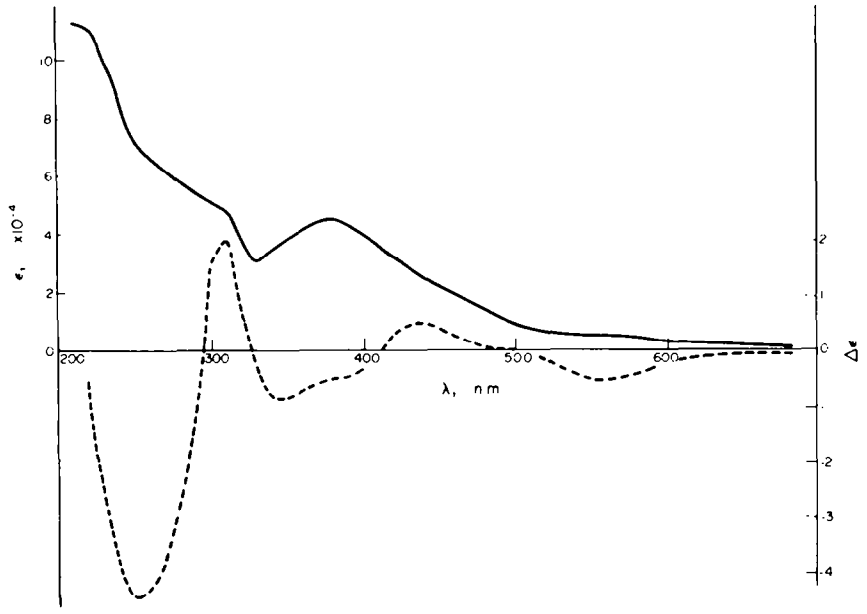
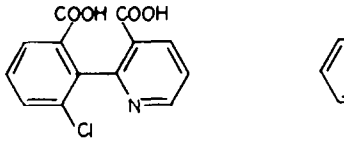
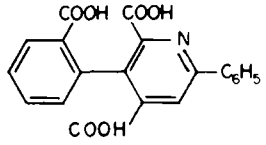


Fig. 2. Isotropic absorption spectrum (——) and circular dichroism (------) of the 2 : 1 molar complex of streptonigrin monoxime (1: X = NOH) with ferrous ion, in ethanol.

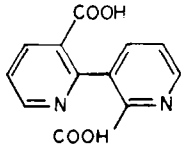
$[\alpha]_D$. The conclusion was that the sugar portion is a derivative of the rare monosaccharide 6-deoxy-L-arabinohexose-2-ulose. The case may however be akin to that of the tannin extracted molecules[12] containing hexahydroxydiphenic acid. For example,[12] corilagin(III) (from dividivi) has $[\alpha]_D^{20} = -246 \pm 2°$ (*c* 1.5, EtOH): it has the β-configuration in structure III. The optical activity of the corilagin arises both from chiral C atoms and the atropisomerism, whereas that of the derived diphenic acid is due to atropisomerism alone. In the same way, in



IVA



IVB



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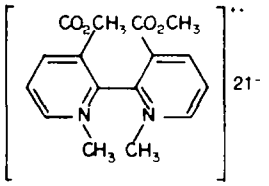
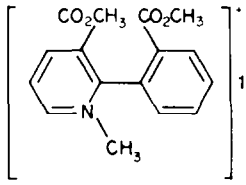
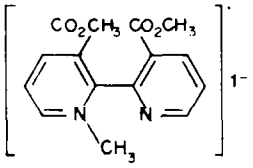


VIA



VIB



VIC

Table 1. Isotropic absorption (AB) and circular dichroism (CD) spectra

Streptonigrin.[a]		Iron-streptonigrinoxime complex.[b]	
AB[b]	CD	AB	CD
λ nm (ε x 10^{-4})	λ nm (Δε)	λ nm (ε x 10^{-4})	λ nm (Δε)
242 (4.18)	240 (d)	225 (10.6)	250 (-4.44)
	270 (-0.5)	305 (4.6) sh	310 (+2)
290 (1.5) sh	290 (+ 1.0)		345 (-0.83)
	325 (-2.5)	375 (4.3)	
375 (1.77)			390 (-0.5)
	400 (-4.72)		430 (+0.5)
	470 (-4.67)		560 (-0.5)
	560 (-3.94) sh		

sh = shoulder

a = measured in ethanol

b = measured in ethanol/water

c = Lit. values (Ref. 19), λ_{max} = 245 ($E^{1\%}_{1cm}$ = 8.25)

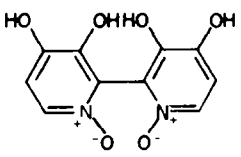
λ_{max} = 375-380 ($E^{1\%}_{1cm}$ = 350)

Molar absorptions were obtained by multiplying these values by the molecular wt. of SN and dividing by 10.

d = not measured because of solvent interferences.

caerulomycin D (IID), the rotation $[\alpha]_D$ will be due to the sugar part and to a contribution from the preferred atropisomer. This latter will, even for a small atropisomeric preference, certainly be large enough to vitiate arguments based on those values of $[\alpha]_D$ which would be appropriate to a molecule with free rotation about the 2,2'-bipyridyl linkage.

Steele and Adams attributed[13] their failure to resolve the phenylpyridines (1VA and 1VB) to ready distortions in the molecule, because of the electrical attraction of the basic nitrogen to the acidic carboxyl group. The existence of atropisomerism in streptonigrin shows that there is no general difficulty with pyridinyl as an aryl residue. Surprisingly, few bipyridyls have been resolved: the 2,3'-dipyridyl-2'3-dicarboxylic acid (V) of Brydowna[14] (via strychnine or quinine) and quaternized 3,3-dicarbomethoxy 2,2'-bipyridinium salts (VI A–C) by Breckenridge.[15]



VII

Also rather surprising is the absence of optical activity in orellanine, a highly toxic principle of the mushroom Cortinarius orellanus Fries, apparently[16] the 2,2'-bipyridyl-N,N'-di-oxide (VII).

We are continuing our work on natural and synthetic optically active 2,2'-bipyridyls and the like, and in particular on their metal-binding properties. Such properties are of interest in the light of the discovery[17] by Lown and Sim that single strand cleavage of PM2 covalently closed circular-DNA induced by reductively activated 5,8-quinolinequinones (as models for streptonigrin) is specifically catalyzed by ferrous and cuprous ions.

EXPERIMENTAL

After attempts to culture bacteria had failed, streptonigrin was a kind gift, through Dr. J. D. Douras of Developmental Therapeutics Programme (NSC 45282), Chemotherapy, NCI, NIH, Bethesda, Maryland, U.S.A.

The purity of streptonigrin was determined by an accurate check of the extinction coefficients (in EtOH) with published values.[19] Streptonigrin monoxime was made by the method[9] of Rao.

Job's plot was done by making equimolar solns of streptonigrin monoxime and Fe(II) in 50% EtOH/water and mixing the two in different proportions, keeping the final volume constant. An aqueous solution of Fe(II) (as sulphate) was added to a soln of streptonigrin monoxime in EtOH to give a green soln. The CD of this soln was determined after 10 mins by which time the soln had come to an equilibrium.

CD measurement utilized the Jouan (Jobin-Yvon) Dichrographe Mark III.

We wish to thank NIH for our sample and SRC for a maintennce grant to Miss Dholakia. Prof. G. Snatzke (University of Bochum) kindly discussed some results with us. Prof. A. Pelter (University College, Swansea) gave us useful help in literature work, as did Prof. A. H. Jackson of this Department, and Dr. J. C. Danilewicz (Pfizer, U.K.) helped us with other enquiries.

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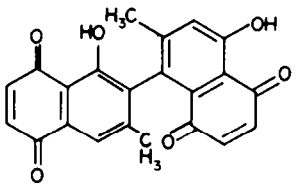
[3]An extensive list is in the thesis of Miss Dholakia, Univ. of Wales, 1981: it includes bridged biaryls and quinones such as isodiospyrin, the binaphthaquinone shown,



isolated from *Diospyros chloroxylon* (Ebenaceae): $[\alpha]_D = 42.7^c$ ($CHCl_3$); *cf* G. S. Sidhu and K. K. Prasad, *Tetrahedron Letters* 2905 (1967); A. L. Fallas and R. H. Thomson, *J. Chem. Soc.* (C), 2279 (1968); K. Yoshihira, M. Tezuka and S. Natori, *Tetrahedron Letters*, 7, (1970).

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[6]A. Moscowitz, *Tetrahedron* **13**, 48 (1960).

[7]Streponigrin possesses in ring A of I (X = O) the aminoquinone moiety which is common to mitomycin, geldanamycin, rifamycin, actinomycin, although these others seem not to have rotations.

[8]This observation supports the mechanism proposed by Lown on the possible metal binding sites during the selective metal complex autoxidation of reduced streptonigrin with the production of oxidizing species. J. W. Lown, *Bioorganic Chem.* (Edited by E. E. Van Tamelen) Vol III. Academic Press, New York (1977).

[9]*Br. Pat.* 1,043,858 (1966) to K. V. Rao of Pfizer.

[10]Actually, the formula drawn for the oxime (Ref. 9) has the N of the derivatives of oxime incorporated in the ring, *at* the 8 position, but from other formulae given (e.g. for the amine produced by reduction of the oxime), this is clearly a printing error.

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[16]W. Z. Antkowiak and W. P. Gessner, *Tetrahedron Letters* **21**, 1931 (1979); orelline (derived from Orellanine either by heating or by catalytic hydrogenation) is said to be 3,4,3',4'-tetrahydroxy-2,2'-bipyridyl, and to form a red complex with Fe^{2+}. However 3,3'-disubstitution of 2,2'-bipyridyls usually renders their ferrous complexes either very unstable or colourless. Red colours with ferrous ions are not unique to the 1,2-di-imines, but also, for example arise in the Skraup test for α-picolinic acids. While 3,3'-substituted 2,2'-bipyridyl-N,N'-dioxides seem not to have been resolved directly, H. Kanno, K. Kashiwabara and J. Fujita, *Bull. Chem. Soc. Japan* **52**, 1408 (1979) made the tris-complex of Cr(III) with 3,3'-dimethyl-2,2'-bipyridyl-N,N'-dioxide (L), resolved this into enantiomers, and decomposed $[Cr(S\text{-}L)_3]^{3+}$ and $[Cr(R\text{-}L)_3]^{3+}$ yielding (+) and (−) 3,3'-dimethyl-2,2'-bipyridyl-N,N'-dioxides. Orellanine might be expected to be about as stable to racemization.

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[18]E. L. Eliel, *Stereochemistry of Carbon Compounds*. McGraw-Hill, New York (1962).

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