ALBOCYCLINE: STRUCTURE DETERMINATION BY X-RAY CRYSTALLOGRAPHY

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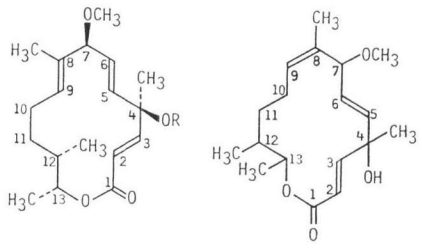
The structure and absolute configuration of the macrolide antibiotic albocycline (**1a**) has been determined by X-ray crystallographic analysis on the derived *p*-bromobenzoate (**1b**). The absolute configuration of albocycline is 4*R*, 7*S*, 12*S*, 13*R*.

Albocycline (**1a**) is a macrolide antibiotic with a 14-membered macrocyclic ring which is lacking any carbohydrate substituent. The antibiotic activity of albocycline is limited, with *in vitro* activity primarily *versus* staphylococci, through inhibition of nicotinate biosynthesis[1]. Albocycline was isolated from three strains of *Streptomyces* at The Tanabe Seiyaku Company[2] as well as from *Streptomyces maizeus* at The Upjohn Company.[3]

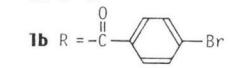
In a series of papers[4~7], the Japanese authors described the chemical and spectroscopic studies which they used to elucidate the gross chemical structure of the molecule, which they assigned as **2**.

By analysis of ozonolysis and hydrogenation products they were able to correctly determine the location of the substituents on the macrocyclic ring. They assigned the *E*-stereochemistry to the 2,3 and 5,6-double bonds based on proton NMR coupling constants, but lacking such a spectral handle they arbitrarily assigned the wrong stereochemistry to the 8,9-double bond and were unable to assign the relative or absolute stereochemistry of the four asymmetric centers.



1a R = H

2



1b R = –C(=O)–C$_6$H$_4$–Br

As part of our efforts aimed at chemical modification of antibiotics, we elected to examine albocycline as a possible candidate for an analog program. As the first step in this process, we wished to confirm the structure proposed in the literature and to define all of the relevant stereochemistry. The results of these studies are presented in this paper.

Experimental

^{13}C NMR spectra were recorded on a Varian FT-80A spectrometer in the indicated solvent. Chemical shifts are reported in ppm (δ) downfield from internal TMS standard. ^{1}H NMR spectra were recorded on a Varian EM-390 spectrometer in the indicated solvent. Chemical shifts are reported in ppm (δ) downfield from internal TMS. Spin multiplicities are designated s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 257 spectrophotometer.

Albocycline *p*-Bromobenzoate (**1b**)

To a solution of 3.0 g (9.7 mmole) of albocycline (**1a**) in 40 ml of dry pyridine was added 2.2 g (10.0 mmole) of *p*-bromobenzoyl chloride and 100 mg (0.82 mmole) of 4-dimethylaminopyridine. The mixture was heated to 80°C under a nitrogen atmosphere for 70 hours, cooled and the pyridine was removed *in vacuo*. The residue was partitioned between 200 ml of hexane and 100 ml of H_2O. The aqueous phase and some insoluble material were removed and the organic layer was washed with 100 ml of H_2O, 100 ml of brine and dried with $MgSO_4$. Removal of solvent *in vacuo* gave 3.59 g of crude yellow glass. The material was purified by filtration through 200 g of silica gel packed with hexane and eluted with 500 ml of 5% AcOEt-hexane, 1 liter of 10% AcOEt-hexane and 1 liter of 15% AcOEt-hexane taking 200 ml cuts. Fractions 4, 5 and 6 were pooled on the basis of TLC to give, after removal of solvent, 2.54 g (5.17 mmole, 53%) of product as a colorless glass which solidified on standing. Material for analysis and X-ray studies was further purified by preparative thin-layer chromatography on silica gel (25% AcOEt-hexane) and recrystallization from *n*-hexane to give colorless crystals: m.p. 90~92.5°C; ^{13}C NMR ($CDCl_3$) δ 165.6, 164.4, 151.2, 136.0, 133.3, 131.8, 131.1, 130.8, 129.6, 129.3, 128.2, 115.8, 83.7, 81.0, 75.6, 57.2, 39.9, 34.2, 25.1, 22.9, 18.3, 16.2 and 14.7; ^1H NMR ($CDCl_3$) δ 7.83 (A of AB, J_{AB}=9 Hz, 2, aromatic), 7.50 (B of AB, J_{AB}=9 Hz, 2, aromatic), 6.75 (A of AB, J_{AB}=15 Hz, 1, olefinic), 5.87 (B of AB, J_{AB}=15 Hz, 1, olefinic), 5.85 (A of ABX, J_{AB}=17 Hz, 1, olefinic), 5.47 (B of ABX, J_{AB}=17 Hz, J_{BX}=5 Hz, 1, olefinic), 5.18 (t, J=6 Hz, 1, olefinic), 4.54 (d of q, J_1=6 Hz, J_2=2 Hz, 1, –C*H*-CH_3O–), 3.98 (X of ABX, J_{BX}=5 Hz, 1, –C*H*OCH_3), 3.24 (s, 3, OCH_3), 1.84 (s, 3, CH_3), 1.60 (s, 3, CH_3), 1.18 (d, J=7 Hz, 3, CH_3), 0.85 (d, J=7 Hz, 3, CH_3), 1.0~2.3 (m, –CH–, –CH_2–); IR (KBr) 2980, 2920, 2825, 1720, 1595, 1300, 1260, 1240, 1200, 1180, 1110, 1090, 995, 780 cm^{-1}; $[\alpha]_D$ −90° (*c* 0.894, $CHCl_3$).

Anal. Calcd. for $C_{25}H_{31}O_5Br$: C 61.10, H 6.36, Br 16.26.
Found: C 60.93, H 6.31, Br 16.04.

X-Ray Crystallography

Crystal data for albocycline *p*-bromobenzoate (**1b**), $C_{25}H_{31}O_5Br$, were: monoclinic; space group $P2_1$; Z=2; a=6.728(2)Å; b=8.776(1)Å; c=20.841(1)Å; β=91.30(1)°; D_{calc}=1.33 g cm^{-3}; μ(CuK)=23.6 cm^{-1}; 2042 reflections, of which 1968 had intensities greater than 3 standard deviations. Low temperature (−150°C) intensity data for all reflections with $2\theta<138°$ were collected using the step-scan technique on a Syntex $P2_1$ diffractometer controlled by a Harris computer using graphite monochromatized CuKα radiation (λ=1.5418Å). The data were corrected for systematic errors, including absorption.[8] The usual Lorentz correction was made along with a polarization correction appropriate for a monochromator with 50% perfect character. Standard deviations in observed intensities were approximated by the function $\sigma^2(I)=\sigma^2$ counting statistics$+(0.033I)^2$, where the coefficient of I was calculated from intensities of ten reflections monitored throughout the data collection, considering deviations in intensities which were not explained by counting statistics.[9]

The structure was solved by direct methods using DIREC* and refined (including coordinates and anisotropic thermal parameters of heavier atoms and hydrogen coordinates) by multiple matrix crystallographic least squares minimizing the function $\sum w(|F_o|^2-|F_c|^2)^2$ where weights w were taken as the reciprocals of the variances $\sigma^2(F_o^2)$. The absolute configuration was determined by comparison of accurate measurements on the 8 reflections most affected by a change in enantiomer, using the method of BIJVOET.[10] All 8 reflections (32 measurements) indicated the enantiomer reported.

Atomic form factors are from "International Tables for X-ray Crystallography"[11], except for hydrogen form factors which are taken from STEWART, DAVIDSON and SIMPSON.[12] The final agreement index R[$R=\sum||F_o|-|F_c||/\sum|F_o|$] was 0.035. All calculations were carried out on an IBM 370 computer using the CRYM system of crystallographic programs.

Results and Discussion

To facilitate the X-ray studies we wished to prepare a derivative of the parent compound which would

* DIREC, a direct methods computer program which uses quartets, and the CRYM system of crystallographic programs, were written by DAVID J. DUCHAMP, The Upjohn Company, Kalamazoo, MI.

contain a heavy atom and which would crystallize readily. Esterification of the hydroxyl group was the obvious choice for preparation of a derivative while minimizing changes in the structure, and we therefore chose to prepare the corresponding *p*-bromobenzoate (**1b**).

The preparation of albocycline acetate, in low yield, has been reported in the literature.[3,4] Using modified conditions, we were able to prepare the acetate in quantitative yield. Analogous derivatization with *p*-bromobenzoyl chloride (pyridine, 4-dimethylaminopyridine, 80°C) was much slower, however, and only a 53% yield of the ester was realized. Recrystallization of the albocycline *p*-bromobenzoate was hampered by the high solubility of the compound in most organic solvents, but crystals suitable for the X-ray study were finally obtained from concentrated hexane solutions. Analysis of **1b** by a variety of spectral methods proved that the substrate had not undergone any unexpected structural changes during the esterification process.

Fig. 1 shows the conformation and absolute configuration of **1b**, which is 4*R*, 7*S*, 12*S*, 13*R*. Numbering and ring torsion angles are also shown. The final coordinates are given in Table 1. Bond lengths, listed in Table 2, are close to expected values. Double bonds are localized except for the conjugated C2=C3 and C1=O1 bonds, which cause the C1–C2 bond to be somewhat shortened. There are few close intermolecular contacts. Only 4 non-hydrogen intermolecular distances are less than 3.5 Å, and these are the only contacts close to the sum of the van der Waals radii. O1 is 3.17 Å from Br in the molecule related by 1−x, y−1/2, 1−z; and the other 3 contacts are with the molecule related by x−1, y+1, z: C20–O1, 3.38 Å; O14–C13, 3.29 Å; and O14–C13M, 3.37 Å.

Fig. 2 is an edge-on view of the molecule showing that the 14-membered ring is quite flat. The me-

Fig. 1. A view of *p*-bromobenzoylalbocycline drawn from three-dimensional coordinates. Torsion angles internal to the ring are in degrees.

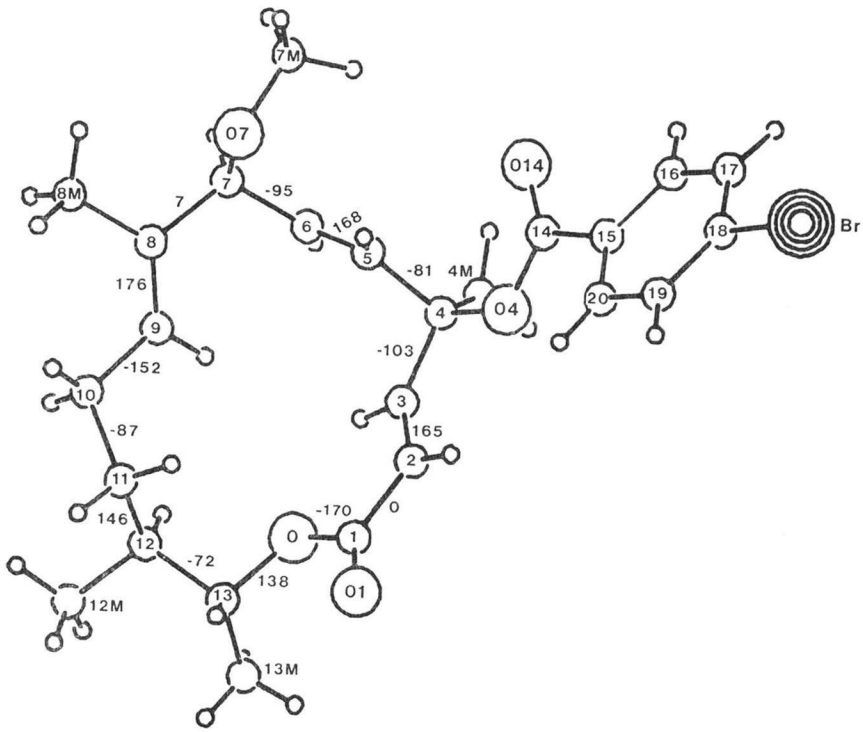


Table 1. Fractional atomic coordinates ($\times 10^4$) and standard deviations for *p*-bromobenzoylalbocycline.

	x	y	z
Br	902 (1)	4058	5442 (1)
O	13697 (5)	564 (3)	2681 (1)
C (1)	12511 (7)	435 (5)	3189 (2)
O (1)	12501 (5)	−660 (4)	3539 (1)
C (2)	11145 (7)	1749 (5)	3239 (2)
C (3)	11181 (7)	2891 (4)	2814 (2)
C (4)	9642 (6)	4109 (6)	2707 (1)
C (4M)	10586 (8)	5690 (5)	2664 (2)
O (4)	8306 (4)	4022 (4)	3252 (1)
C (5)	8543 (7)	3609 (4)	2101 (2)
C (6)	9184 (7)	3846 (4)	1513 (2)
C (7)	8270 (7)	3085 (5)	925 (2)
O (7)	6241 (5)	2675 (3)	1011 (1)
C (7M)	4944 (8)	3951 (7)	1007 (3)
C (8)	9336 (7)	1633 (5)	726 (2)
C (8M)	8512 (9)	952 (6)	107 (2)
C (9)	10780 (7)	1006 (5)	1070 (2)
C (10)	11815 (8)	−473 (5)	927 (2)
C (11)	12628 (7)	−1260 (5)	1539 (2)
C (12)	14700 (6)	−734 (5)	1752 (2)
C (12M)	16306 (9)	−1714 (6)	1444 (2)
C (13)	14917 (7)	−733 (5)	2488 (2)
C (13M)	16964 (8)	−484 (5)	2761 (2)
C (14)	6655 (7)	4864 (5)	3224 (2)
O (14)	6205 (5)	5717 (4)	2785 (1)
C (15)	5363 (7)	4635 (4)	3793 (2)
C (16)	5390 (8)	3279 (6)	4137 (2)
C (17)	4018 (8)	3083 (6)	4631 (2)
C (18)	2754 (6)	4265 (6)	4776 (2)
C (19)	2766 (7)	5636 (5)	4445 (2)
C (20)	4093 (7)	5812 (5)	3947 (2)

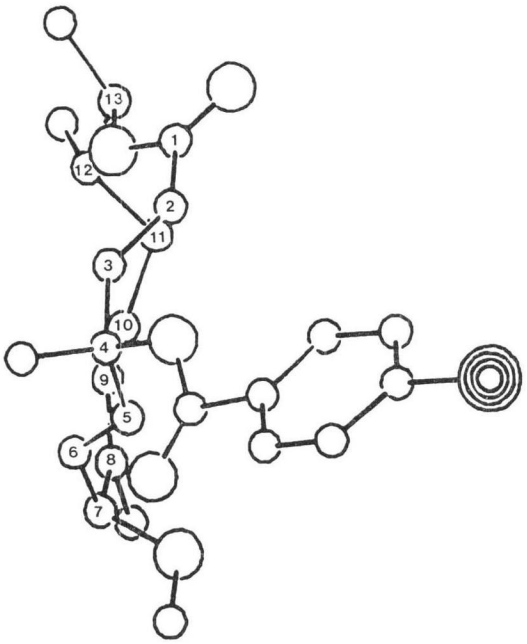
Table 2. Bond lengths in Å and standard deviations for *p*-bromobenzoylalbocycline.

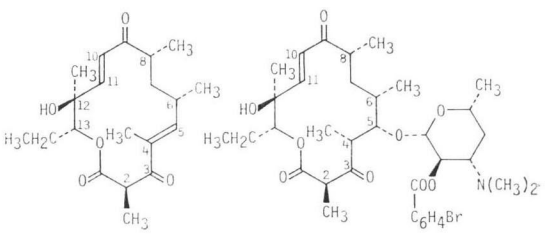
Br	C18	1.895 (4)
O	C1	1.345 (5)
O	C13	1.465 (5)
C1	O1	1.207 (5)
C1	C2	1.480 (6)
C2	C3	1.338 (6)
C3	C4	1.501 (6)
C4	C4M	1.530 (6)
C4	O4	1.466 (4)
C4	C5	1.515 (5)
O4	C14	1.334 (5)
C5	C6	1.325 (5)
C6	C7	1.513 (5)
C7	O7	1.427 (6)
C7	C8	1.525 (6)
O7	C7M	1.420 (7)
C8	C8M	1.516 (7)
C8	C9	1.314 (6)
C9	C10	1.506 (6)
C10	C11	1.540 (6)
C11	C12	1.525 (6)
C12	C12M	1.534 (7)
C12	C13	1.538 (5)
C13	C13M	1.494 (7)
C14	O14	1.215 (5)
C14	C15	1.499 (6)
C15	C16	1.389 (6)
C15	C20	1.383 (6)
C16	C17	1.409 (7)
C17	C18	1.380 (7)
C18	C19	1.387 (7)
C19	C20	1.394 (6)

thyl substituents at 12 and 13 and the *p*-bromobenzoyloxy group at 4 are quasi-equatorial; and the methyl at 4 and the *O*-methyl at 7 are quasi-axial. The three double bonds in the ring, 2=3; 5=6; and 8=9, are all *trans*.

The conformation of the ring in albocycline *p*-bromobenzoate is quite different from that of 14-membered ring macrolides without ring double bonds such as erythromycin[13,14], megalomicin[15], oleandomycin[16], and a lankamycin-related antibiotic, 23672RP[17], although among these other macrolides the ring conformations are quite similar. The albocycline *p*-bromobenzoate ring conformation is most like that of kromycin[18] (**3**) which has two double bonds in the ring. *p*-Bromobenzoylpikromycin[10] (**4**) is structurally very much like kromycin, differing only in that the 4–5 bond is a single bond and in the substituent at 5. However, the ring in *p*-bromobenzoylpikromycin does not resemble the kromycin ring as much as the albocycline ring does, despite the fact that albocycline has different substituents and different sp^2 or sp^3 character at several atoms.

Fig. 2. *p*-Bromobenzoylalbocycline.
Hydrogen atoms were omitted for clarity.

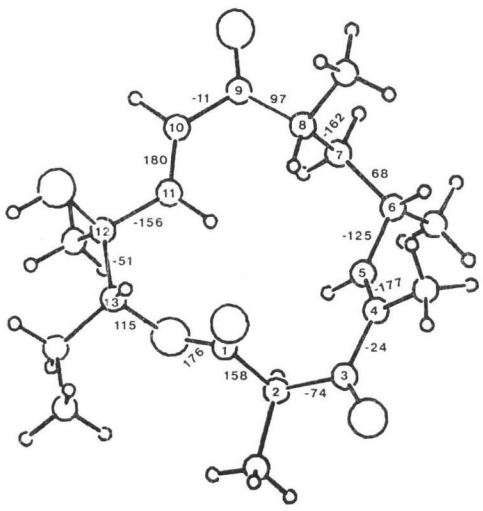




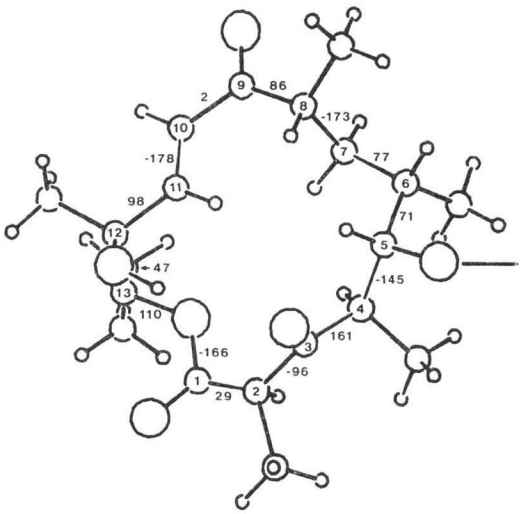
3 4

In order to compare the three structures, Figs. 3 and 4 have been drawn to show kromycin and *p*-bromobenzoylpikromycin in views similar to the views of *p*-bromobenzoylalbocycline in Figs. 1 and 2. The correspondence between kromycin and albocycline is as follows: if the conjugated C3=O3 and C4=C5 bonds in kromycin overlay the C1=O1 and C2=C3 bonds in albocycline, then the kromycin C10=C11 bond also overlays the albocycline C8=C9 bond. The C7–C8=C9–C10 atoms of albocycline define a plane that is approximately the plane of the ring, and similarly, the plane of the kromycin ring is approximately that of atoms C9–C10=C11–C12. Comparing the edge views in Figs. 2 and 4, it can be seen that albocycline atoms 5 and 6 are mirror images through this ring plane of kromycin atoms 7 and 8. Probably atoms 5 and 6 prefer to be in this conformation in the albocycline molecule because an undesirable interaction between the hydrogen at 5 and the methyl group at y is there by avoided. *p*-

Fig. 3. Views drawn from three-dimensional coordinates showing torsion angles in degrees.
The *p*-bromobenzoyl desosamine substituent in *p*-bromobenzoylpikromycin was omitted for clarity.

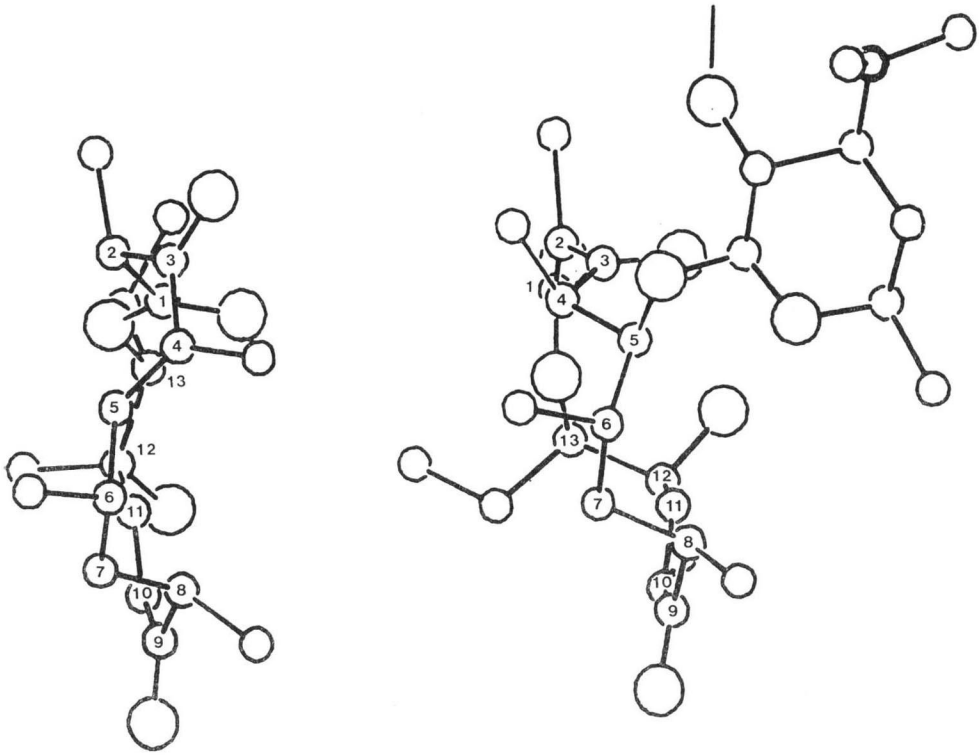


Kromycin[18]



p-Bromobenzoylpikromycin[19]

Fig. 4. Views drawn from three-dimensional coordinates.
Hydrogen atoms and the $-COC_6H_4Br$ group in *p*-bromobenzoylpikromycin were omitted for clarity.



Kromycin *p*-Bromobenzoylpikromycin

Bromobenzoylpikromycin has a ring conformation similar to that of kromycin for atoms 5~12 and quite different for the rest of the ring. As the edge views in Fig. 4 show, the ring is much less flat than the kromycin and albocycline rings.

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