a 5-mm thickness of SilicAR 7GF). The plates were developed ascending in a toluene–ethyl acetate solvent system (9:1, v/v), allowing the solvent to run the entire length (15 mm) of the plate. The plates were then air dried with the assistance of an infrared lamp, and the foregoing procedure was repeated two more times resulting in the separation of two major components which were detected by an ultraviolet mineralight source (254 mμ). The slower moving band was removed and extracted with hot ethyl acetate (400 ml). The adsorbent was removed by filtration and the filtrate concentrated *in vacuo* to a syrup. This syrup was dissolved in 10 ml of absolute acetone and refrigerated at −25° for 1 week, yielding 210 mg of product. Recrystallization from absolute acetone gave an analytical sample of 2,6-dichloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α-D-ribopyranosyl)purine (VI), mp 224–225°, $[\alpha]^{26}D$ +27.0° (*c* 0.5, ethyl acetate). The remaining filtrate was concentrated *in vacuo* to a small volume and refrigerated at −25°, yielding 250 mg of nucleoside material. Recrystallization from absolute acetone furnished an analytical sample of 2,6-dichloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-β-D-ribopyranosyl)purine (IV), mp 185–186°, $[\alpha]^{26}D$ −12.5° (*c* 1.0, ethanol).

Anal. Calcd for $C_{14}H_{14}Cl_2N_4O_5$: C, 43.19; H, 3.60; N, 14.40. Found α-anomer: C, 43.14; H, 3.57; N, 14.46. Found β-anomer: C, 43.08; H, 3.74; N, 14.57.

Method B. A mixture of 2.60 g of finely powdered 1,3,4-tri-*O*-acetyl-2-deoxy-β-D-ribopyranose and 1.90 g of 2,6-dichloropurine (II) in a 25-ml, round-bottomed flask was heated in an oil bath to 120°. To this preheated reaction mixture was added 20 mg of *p*-toluenesulfonic acid, and a water aspirator was attached to the flask. Upon addition of the acid catalyst, the mixture began to evolve acetic acid and gradually formed a clear green melt. When no further evolution of acetic acid was observed (10 min), the flask was removed from the oil bath and its contents dissolved in 200 ml of warm ethyl acetate. The ethyl acetate solution was cooled to 0° by the addition of ice, washed with cold saturated sodium carbonate (three 100-ml portions) and water (two 100-ml portions), and dried over sodium sulfate. The sodium sulfate was removed by filtration and the filtrate concentrated *in vacuo* until a thick syrup remained. This syrup was dissolved in warm acetone; the acetone solution was treated with Norit and then concentrated to 20 ml to yield 1.2 g of nucleoside material, mp 225–230°. Recrystallization of this crude product from acetone gave a pure sample of 2,6-dichloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α-D-ribopyranosyl)purine (VI), mp 228–229°, $[\alpha]^{26}D$ +27.0° (*c* 0.5, ethyl acetate).

Anal. Calcd for $C_{14}H_{14}Cl_2N_4O_5$: C, 43.20; H, 3.60; N, 14.40. Found: C, 43.30; H, 3.90; N, 14.20.

Concentration of the original acetone filtrate to *ca.* 10 ml and allowing the solution to stand at −10° for 24 hr provided an additional crystalline material, mp 170–178°. Recrystallization of this material from small amounts of acetone gave an analytical sample of 2,6-dichloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-β-D-ribopyranosyl)-purine (IV), mp 186–187°, $[\alpha]^{26}D$ −12.5° (*c* 1.0, ethanol).

Anal. Calcd for $C_{14}H_{14}Cl_2N_4O_5$: C, 43.20; H, 3.60; N, 14.40. Found: C, 43.29; H, 3.81; N, 14.70.

6-Amino-2-chloro-9-(2′-deoxy-α-D-ribopyranosyl)purine (XI). To 60 ml of anhydrous methanol was added 1.0 g (0.003 mole) of 2,6-dichloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α-D-ribopyranosyl)-purine, and this mixture was cooled to 5° and saturated with dry ammonia for 1 hr. The resulting solution was sealed in a pressure bottle and allowed to remain at room temperature for 1 week. Excess ammonia was removed in a stream of nitrogen and the solvent removed *in vacuo* at room temperature. The residue was triturated with chloroform (500 ml) overnight and the resulting solid collected by filtration and air dried, yielding 550 mg (73.3%). Three recrystallizations from absolute ethanol gave pure 6-amino-2-chloro-9-(2′-deoxy-α-D-ribopyranosyl)purine (XI).

Anal. Calcd for $C_{10}H_{12}ClN_5O_3$: C, 42.03; H, 4.20; N, 24.50. Found: C, 42.26; H, 4.43; N, 24.26.

Dehalogenation of 6-Amino-2-chloro-9-(2′-deoxy-α-D-ribopyranosyl)purine (XI). 6-Amino-2-chloro-9-(2′-deoxy-α-D-ribopyranosyl)-purine (10 mg) together with 10 mg of 5% palladium-on-carbon catalyst was added to 50 ml of water. The pH of the mixture was adjusted to 8.0 with dilute NaOH and then hydrogenated at 45 psi hydrogen pressure for 2.5 hr at room temperatue. The catalyst was removed by filtration and the filtrate concentrated *in vacuo* to a small volume. The ultraviolet spectrum of the filtrate was identical with that of 6-amino-9-(2′-deoxy-α-D-ribopyranosyl)-purine. Paper chromatograms of the filtrate in three solvent systems revealed R_{Ad} values (A,B,C) which were identical with those observed for 6-amino-9-(2′-deoxy-α-D-ribopyranosyl)purine (VIII), but were significantly different from the R_{Ad} values (A,B,C) observed for 6-amino-9-(2′-deoxy-β-D-ribopyranosyl)purine (VII).

The Absolute Configuration of Caldariomycin

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Abstract: The absolute configuration of the chlorine-containing mold metabolite caldariomycin has been established as *S*, both by X-ray single crystal structure analysis of the bis[(+)-3-bromocamphor-9-sulfonate] and by application of the α-phenylbutyric anhydride method.

The mold metabolite caldariomycin, from *Caldariomyces fumago*, was first isolated by Raistrick, *et al.*,[1] who assigned it the structure 2,2-dichlorocyclopentane-1,3-diol. This has now been confirmed twice by synthesis,[2,3] and the biosynthesis of the compound has also been studied.[4] Caldariomycin is optically active, but the absolute configuration of the two asymmetric centers, which must be the same, has not been reported.

A simple empirical procedure for determining the absolute configuration is the method of Horeau,[5] employing the anhydride of racemic α-phenylbutyric acid. This method appeared to be well suited to the problem of caldariomycin, since the two groups flanking the asymmetric carbon atoms, $-CCl_2-$ and $-CH_2-$, differ considerably in size.

On the other hand, the instability of caldariomycin to alkali required a modification of the usual Horeau procedure, in which the alcohol and anhydride react in pyridine solution, the excess anhydride is decomposed

(1) P. W. Clutterbuck, S. L. Mukhopadhyay, A. E. Oxford, and H. Raistrick, *Biochem. J.*, **34**, 663 (1940).

(2) J. R. Beckwith and L. P. Hager, *J. Org. Chem.*, **26**, 5206 (1961).

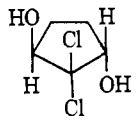
(3) A. W. Burgstahler, T. B. Lewis, and M. O. Abdel-Rahman, *ibid.*, **31**, 3516 (1966).

(4) P. D. Shaw, J. R. Beckwith, and L. P. Hager, *J. Biol. Chem.*, **234**, 2560 (1959).

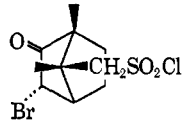
(5) (a) A. Horeau, *Tetrahedron Letters*, 506 (1961); (b) *ibid.*, 965 (1962).

with water, the liberated acids are neutralized with aqueous sodium hydroxide, and the acid is extracted into benzene after acidification.

In the present procedure, the reaction was carried out in a benzene–pyridine mixture, then diluted with water and ether; the pyridine was removed by washing the solution with hydrochloric acid. The phenylbutyric acids were extracted with saturated sodium bicarbonate solution, and then were reextracted upon acidification. This method gave satisfactory results with (−)-menthol. The reaction was carried out on caldariomycin using twice the theoretical amount of the anhydride. The α-phenylbutyric acid isolated was levorotatory, indicating that the absolute configuration at both the asymmetric centers is *S*, as shown in I.



I



II

Since this was the first application of Horeau's method to an alcohol with an adjacent, highly polar, dichloromethylene group, it was considered desirable to confirm (or refute) the results by X-ray analysis of a derivative prepared from a compound with asymmetric centers of known absolute configuration. This could be achieved most simply by esterifying one or both hydroxyl groups with an acid. The bis[(+)-10-camphorsulfonate] of caldariomycin was reported earlier[2] and the crystalline mono[(+)-10-camphorsulfonate] was prepared as a model compound in the present study from reaction of molar proportions of caldariomycin and (+)-10-camphorsulfonyl chloride in benzene–pyridine solution at room temperature.

For application of the heavy atom method[6] the diester of (+)-3-bromocamphor-9-sulfonyl chloride (II) proved superior. This derivative was prepared by a procedure similar to that employed earlier[2] for the bis-[(+)-10-camphorsulfonate]. Since the absolute configuration of (+)-3-bromocamphor-9-sulfonic acid, prepared from (+)-camphor, is known (II),[7] the assignment of relative stereochemistry to the derivative also assigns its absolute configuration; crystal data: $C_{25}H_{34}Br_2Cl_2O_8S_2$, M = 757.4, monoclinic, a = 20.92 ±0.04, b = 10.61 ± 0.02, c = 7.14 ± 0.02 A, β = 112° 15′ ± 15′, V = 1466.7 × 10^{-24} cm³, ρ_{meas} = 1.67 g cm^{-3}, Z = 2, ρ_{calcd} = 1.71 g cm^{-3}. Systematic absences, $0k0$ when $k = 2n + 1$, and the asymmetric nature of the molecule established the space group as $P2_1(C_2^2)$; linear absorption coefficient, μ (Cu Kα) = 72.3 cm^{-1}.

Photographic data collected from three crystals mounted about the *c* axis provided a total of 1259 independent nonzero structure amplitudes. The structure was solved by the heavy atom method[6] and refined by least-squares methods to a final *R* factor of 0.12.[8]

$$R = \Sigma||F_{obsd}| - |F_{calcd}||/\Sigma F_{obsd}$$

(6) J. M. Robertson and I. Woodward, *J. Chem. Soc.*, 219 (1937); 36 (1940).

(7) F. H. Allen and D. Rogers, *Chem. Commun.*, 837 (1966).

(8) The list of h, k, l, F_{obsd}, F_{calcd}, and α_{calcd} is available from the American Documentary Institute, Auxiliary Publications Project, Library of Congress, Washington, D. C. 20542, Document No. 9650. A copy may be secured by citing the document number and by remitting $3.75 for photoprints, or $2.00 for 35-mm microfilm. Advance payment is required. Make checks payable to: Chief, Photoduplication Service, Library of Congress.

Table I. Final Atomic Coordinates and Standard Deviations (in parentheses) as Fractions of the Unit Cell Edge[a]

	x	*y*	*z*	*Bθ*
C(1)	0.6233 (24)	0.284 (5)	0.989 (7)	4.7 (1.0)
C(2)	0.6780 (19)	0.368 (4)	0.109[b] (6)	2.9 (0.8)
C(3)	0.6741 (21)	0.475 (5)	0.977 (7)	3.9 (1.0)
C(4)	0.6183 (28)	0.446 (6)	0.766 (8)	5.6 (1.4)
C(5)	0.6479 (20)	0.319 (4)	0.680 (6)	3.3 (0.8)
C(6)	0.6530 (23)	0.212 (5)	0.837 (7)	5.0 (1.0)
C(7)	0.5704 (20)	0.369 (4)	0.846 (6)	3.4 (0.8)
C(8)	0.5429 (23)	0.468 (5)	0.962 (7)	4.0 (1.1)
C(9)	0.5095 (24)	0.309 (5)	0.692 (7)	3.4 (1.0)
C(10)	0.5995 (25)	0.189 (5)	0.112[b] (8)	5.3 (1.1)
C(11)	0.3038 (18)	0.286 (4)	0.574 (6)	2.6 (0.7)
C(12)	0.3264 (21)	0.379 (4)	0.418 (6)	3.6 (0.9)
C(13)	0.2652 (23)	0.353 (5)	0.210 (7)	4.6 (1.0)
C(14)	0.2006 (24)	0.301 (5)	0.247 (7)	4.2 (1.0)
C(15)	0.2237 (25)	0.325 (5)	0.510 (7)	5.0 (1.1)
O(1)	0.7131 (16)	0.362 (3)	0.291[b] (5)	5.0 (0.7)
O(2)	0.4838 (19)	0.398 (4)	0.320 (6)	6.9 (0.9)
O(3)	0.4425 (16)	0.528 (3)	0.547 (5)	5.7 (0.8)
O(4)	0.3863 (14)	0.320 (3)	0.411 (4)	3.7 (0.6)
S	0.4567 (8)	0.403 (2)	0.481 (3)	[c]
Br	0.7715 (2)	0.498 (1)	0.970 (1)	[c]
Cl(1)	0.3518 (6)	0.339 (2)	0.825 (2)	[c]
Cl(2)	0.3126 (7)	0.136 (2)	0.539 (2)	[c]
C(1′)	0.0625 (23)	0.749 (5)	0.183 (7)	4.0 (1.0)
C(2′)	0.0831 (23)	0.886 (5)	0.190 (8)	4.0 (1.0)
C(3′)	0.1251 (18)	0.915 (4)	0.403 (6)	1.7 (0.8)
C(4′)	0.1135 (21)	0.798 (4)	0.504 (6)	3.5 (0.9)
C(5′)	0.0404 (26)	0.787 (5)	0.502 (8)	5.7 (1.2)
C(6′)	−0.0000 (25)	0.765 (5)	0.248 (7)	5.4 (1.1)
C(7′)	0.1199 (19)	0.695 (4)	0.366 (6)	2.5 (0.8)
C(8′)	0.1884 (25)	0.692 (5)	0.344 (8)	5.0 (1.2)
C(9′)	0.1031 (21)	0.567 (5)	0.403 (7)	3.3 (0.9)
C(10′)	0.0373 (32)	0.692 (7)	−0.041 (10)	6.6 (1.6)
O(1′)	0.0714 (18)	0.955 (4)	0.047 (6)	6.1 (0.9)
O(2′)	0.1908 (16)	0.587 (4)	0.789 (5)	5.6 (0.8)
O(3′)	0.1316 (17)	0.379 (4)	0.674 (5)	5.8 (0.8)
O(4′)	0.2231 (14)	0.452 (3)	0.550 (4)	4.1 (0.6)
S′	0.1601 (8)	0.496 (2)	0.633 (3)	[c]
Br′	0.0988 (3)	0.075[b] (1)	0.485 (1)	[c]

[a] The isotropic temperature factors are in the form of exp $-[B_\theta \sin^2\theta/\lambda^2]$, the anisotropic temperature factors as exp $-[b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl]$. [b] It is necessary to add the unit cell length in this direction to form a complete molecule. [c] Final thermal parameters are

	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	$10^4 b_{23}$
S	13 (5)	253 (38)	165 (58)	−8 (21)	3 (27)	138 (74)
Br	20 (1)	210 (10)	361 (19)	−20 (7)	45 (8)	187 (24)
Cl(1)	34 (4)	145 (17)	162 (34)	−6 (14)	79 (18)	5 (38)
Cl(2)	37 (5)	115 (17)	348 (44)	26 (15)	72 (23)	−11 (44)
S′	26 (5)	54 (19)	340 (60)	6 (21)	123 (29)	−20 (62)
Br′	30 (1)	108 (6)	372 (19)	20 (6)	49 (9)	−51 (20)

The final atomic coordinates and temperature factors are given in Table I together with the estimated standard deviations. A representation of the final electron density map in the region of an entire molecule is shown in Figure 1. Figure 2 shows the structure, stereochemistry, and atom numbering in the caldariomycin bis[(+)-3-bromocamphor-9-sulfonate] molecule. On the basis of the known absolute configuration of (+)-3-bromocamphor,[7] the absolute stereochemistry of caldariomycin is established as I, in agreement with the results from Horeau's method.

The calculated bond lengths and angles are listed in Tables II and III. The standard deviations are very large, probably due to neglect of absorption corrections

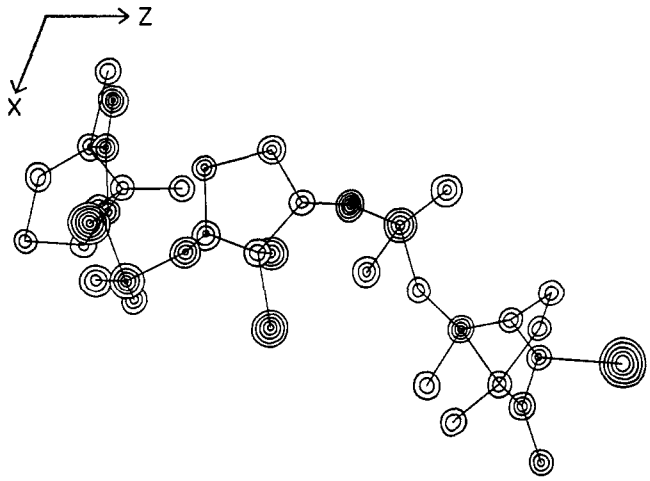


Figure 1. A superimposed contour representation of the final electron density map covering the region of one molecule. Contours are at arbitrary but equal intervals, with every second line drawn in the case of the chlorine and sulfur atoms and every fourth line in the case of the bromine atoms.

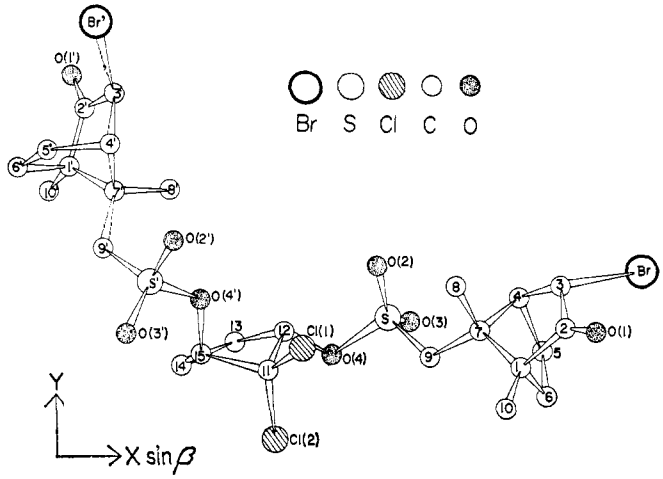


Figure 2. The structure of the caldariomycin bis[(+)-3-bromocamphor-9-sulfonate] molecule showing the atom numbering used in the present study.

on the intensity data and the use of three crystals to record the data, Detailed discussion of the molecular geometry is not therefore justified,

Table II. Bond Distances in Angstrom Units[a]

C(1)–C(2)	1.45	C(12)–O(4)	1.42
C(1)–C(6)	1.63	C(13)–C(14)	1.57
C(1)–C(7)	1.49	C(14)–C(15)	1.77
C(1)–C(10)	1.54	C(15)–O(4′)	1.38
C(2)–C(3)	1.46	C(1′)–C(2′)	1.51
C(2)–O(1)	1.23	C(1′)–C(6′)	1.55
C(3)–C(4)	1.55	C(1′)–C(7′)	1.51
C(3)–Br	2.07	C(1′)–C(10′)	1.60
C(4)–C(5)	1.69	C(2′)–C(3′)	1.47
C(4)–C(7)	1.56	C(2′)–O(1′)	1.20
C(5)–C(6)	1.57	C(3′)–C(4′)	1.50
C(7)–C(8)	1.57	C(3′)–Br′	1.94
C(7)–C(9)	1.48	C(4′)–C(5′)	1.53
C(9)–S	1.80	C(4′)–C(7′)	1.51
S–O(2)	1.46	C(5′)–C(6′)	1.70
S–O(3)	1.47	C(7′)–C(8′)	1.50
S–O(4)	1.62	C(7′)–C(9′)	1.45
C(11)–C(12)	1.68	C(9′)–S′	1.79
C(11)–C(15)	1.62	S′–O(2′)	1.43
C(11)–Cl(1)	1.78	S′–O(3′)	1.45
C(11)–Cl(2)	1.63	S′–O(4′)	1.70
C(12)–C(13)	1.58		

[a] The average standard deviation for a C–Br bond is ±0.04, for S–O, S–C, Cl–C bonds is ±0.05, and for C–C and C–O bonds is ±0.06 A.

Table III. Bond Angles in Degrees[a]

C(2)C(1)C(6)	106	C(7)C(9)S	118
C(2)C(1)C(7)	104	C(9)SO(2)	110
C(2)C(1)C(10)	115	C(9)SO(3)	111
C(6)C(1)C(7)	101	C(9)SO(4)	99
C(6)C(1)C(10)	111	O(2)SO(3)	118
C(7)C(1)C(10)	118	O(2)SO(4)	108
C(1)C(2)C(3)	105	O(3)SO(4)	109
C(1)C(2)O(1)	128	C(12)O(4)S	117
C(3)C(2)O(1)	126	C(12)C(11)C(15)	101
C(2)C(3)C(4)	108	C(12)C(11)Cl(1)	107
C(2)C(3)Br	107	C(12)C(11)Cl(2)	113
C(4)C(3)Br	113	C(15)C(11)Cl(1)	110
C(3)C(4)C(5)	105	C(15)C(11)Cl(2)	112
C(3)C(4)C(7)	96	Cl(1)C(11)Cl(2)	114
C(5)C(4)C(7)	95	C(11)C(12)C(13)	101
C(4)C(5)C(6)	105	C(11)C(12)O(4)	104
C(1)C(6)C(5)	101	C(13)C(12)O(4)	108
C(1)C(7)C(4)	99	C(12)C(13)C(14)	110
C(1)C(7)C(8)	112	C(13)C(14)C(15)	103
C(1)C(7)C(9)	117	C(11)C(15)C(14)	96
C(4)C(7)C(8)	105	C(11)C(15)O(4′)	106
C(4)C(7)C(9)	117	C(14)C(15)O(4′)	110
C(8)C(7)C(9)	107	C(15)O(4′)S′	114
C(2′)C(1′)C(6′)	98	C(4′)C(5′)C(6′)	97
C(2′)C(1′)C(7′)	102	C(1′)C(6′)C(5′)	101
C(2′)C(1′)C(10′)	112	C(1′)C(7′)C(4′)	93
C(6′)C(1′)C(7′)	105	C(1′)C(7′)C(8′)	113
C(6′)C(1′)C(10′)	110	C(1′)C(7′)C(9′)	110
C(7′)C(1′)C(10′)	125	C(4′)C(7′)C(8′)	114
C(1′)C(2′)C(3′)	107	C(4′)C(7′)C(9′)	118
C(1′)C(2′)O(1′)	126	C(8′)C(7′)C(9′)	108
C(3′)C(2′)O(1′)	127	C(7′)C(9′)S′	116
C(2′)C(3′)C(4′)	100	C(9′)S′O(2′)	112
C(2′)C(3′)Br′	112	C(9′)S′O(3′)	111
C(4′)C(3′)Br′	118	C(9′)S′O(4′)	98
C(3′)C(4′)C(5′)	114	O(2′)S′O(3′)	121
C(3′)C(4′)C(7′)	102	O(2′)S′O(4′)	106
C(5′)C(4′)C(7′)	106	O(3′)S′O(4′)	105

[a] Standard deviations range from ±1.5 to ±3.0°.

Table IV. Intermolecular Contacts ≤3.70 A

C(5′)···O(3′)[a]	3.47
C(6′)···O(3′)[a]	3.24
Br′···C(5′)[a]	3.71
O(1′)···C(10′)[b]	3.38
Br′···C(10′)[b]	3.59
C(4)···Cl(2)[c]	3.64
O(3)···C(5)[c]	3.66
O(3)···C(6)[c]	3.35
Br···Cl(2)[c]	3.69
O(1′)···Br[c]	3.37
C(8)···O(2)[d]	3.32
O(1)···C(5)[d]	3.56
Cl(1)···O(2)[d]	3.63
C(5′)···C(10′)[d]	3.44

[a] Molecule related to that in Table I by $-x$, $1/2 + y$, $1 - z$. [b] Molecule related to that in Table I by $-x$, $1/2 + y$, $-z$. [c] Molecule related to that in Table I by $1 - x$, $1/2 + y$, $1 - z$. [d] Molecule related to that in Table I by x, y, $1 + z$.

The packing in the crystal unit cell is shown in Figures 3 and 4. Intermolecular contacts less than 3.70 A are given in Table IV.

Experimental Section[9]

Treatment of (−)-Menthol with the Anhydride of Racemic α-Phenylbutyric Acid. A solution of 0.6 g of the anhydride, 0.16 g of (−)-menthol, 0.5 ml of pyridine, and 5 ml of benzene was kept

(9) Melting points were determined on a Kofler micro hot stage. Infrared spectra were determined on a Perkin-Elmer double grating Infracord, Model 237. Microanalyses were carried out by Mr. J. Nemeth and his associates.

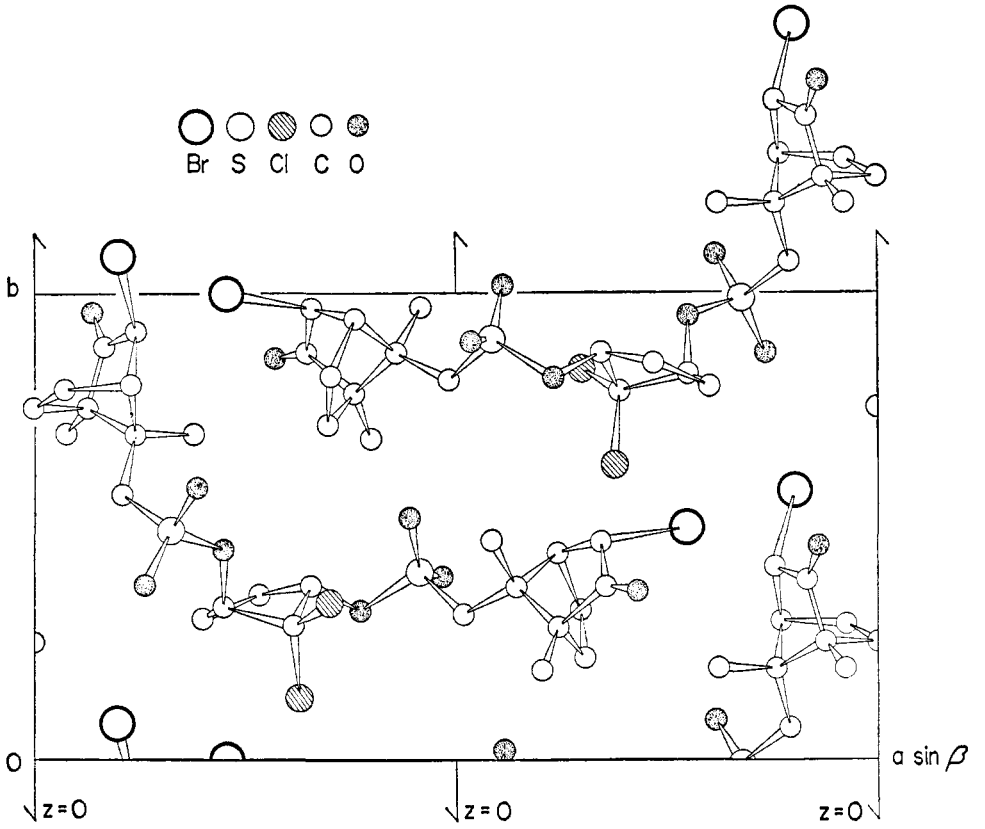


Figure 3. The contents of the unit cell viewed along the *c* axis.

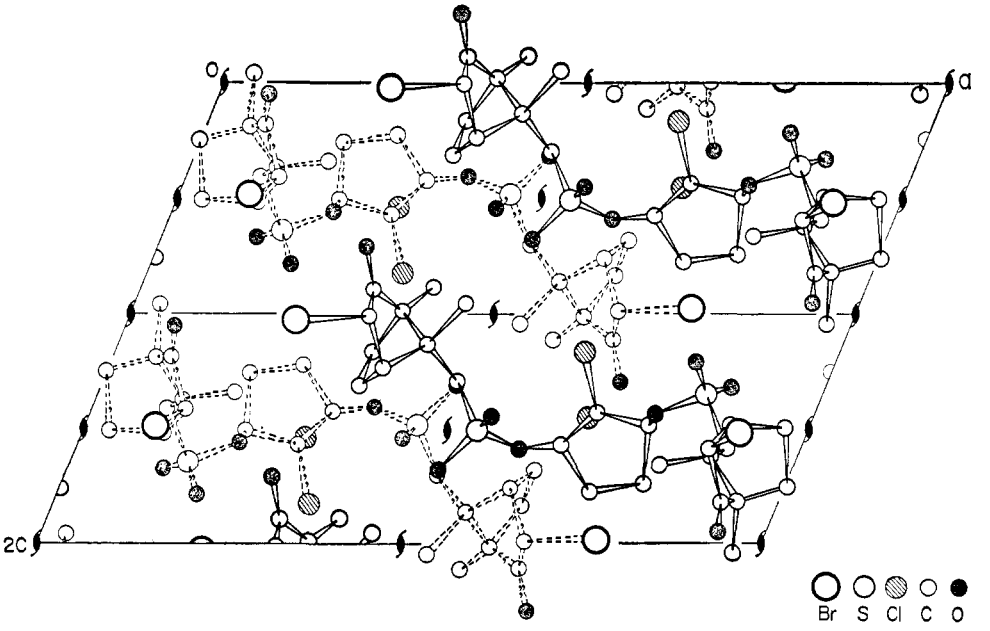


Figure 4. The packing of molecules viewed along the *b* axis. The molecules shown as solid lines lie above those shown as dotted lines.

at room temperature for 23 hr. Water (5 ml) was added, and the mixture was stirred for 30 min, then mixed with 45 ml of ether and with 50 ml of water containing 5 ml of concentrated hydrochloric acid. The ether layer was washed with water, then extracted twice with 25 ml of saturated sodium bicarbonate solution. The bicarbonate solution was acidified with hydrochloric acid and extracted with ether. The ether solution was dried over anhydrous sodium sulfate, and the solvent was removed. The residue dissolved in 5 ml of benzene had α_D +0.46°.

Reaction of Caldariomycin with the Anhydride of Racemic α-Phenylbutyric Acid. This was carried out using 0.6 g of the anhydride, 0.09 g of caldariomycin, 0.5 ml of pyridine, and 5 ml of benzene at room temperature for 24 hr. The reaction mixture was worked up as in the experiment with (−)-menthol. The resultant acid dissolved in 5 ml of benzene had α_D −0.84°.

Reaction of Caldariomycin with (+)-10-Camphorsulfonyl Chloride. A solution of 174 mg of caldariomycin, 250 mg of (+)-10-camphorsulfonyl chloride, 1 ml of pyridine, and 10 ml of benzene was stirred at room temperature for 24 hr. The solution was diluted with 50 ml of ether, washed with saturated salt solution containing 3 ml of concentrated hydrochloric acid, then with three 50-ml portions of saturated salt solution, and dried over anhydrous

sodium sulfate. Removal of solvent gave an oil which was found to be a mixture of three compounds by tlc on silica gel, employing 4:1 benzene–ethyl acetate. These were separated by chromatography over a column (1.8 × 30 cm) of silica gel using the same solvent. The first compound, mp 141°, 85 mg, was identified as caldariomycin bis[(+)-10-camphorsulfonate] (lit. mp 142–143°).[2] Its infrared spectrum contained no hydroxyl absorption.

The second compound, 168 mg, crystallized from benzene–pentane, mp 116°, $[\alpha]^{25}D$ +62.6° (*c* 3.0, benzene). This compound was identified as caldariomycin mono[(+)-10-camphorsulfonate] by hydroxyl absorption at 3580 cm^{-1} in its infrared spectrum.

Anal. Calcd for $C_{15}H_{22}Cl_2O_5S$: Cl, 18.65. Found: Cl, 18.45.

The third compound, 25 mg, was identified as unreacted caldariomycin, by melting point (121–122°),[1] mixture melting point, and tlc.

(+)-3-Bromocamphor-9-sulfonyl Chloride. Thionyl chloride (1.5 ml) was added at 5° with stirring to a solution of 5.6 g of ammonium (+)-3-bromocamphor-9-sulfonate in 30 ml of dry dimethylformamide. The solution was stirred for 1 hr, then diluted with 100 ml of ice cold water. The precipitated solids were filtered off; crystallization from benzene–pentane gave 1.6 g of the acid chloride, mp 136–138° (lit.[10] mp 136–137°). The crystals belong to the orthorhombic system, with approximate parameters, a = 11.03, b = 11.63, and c = 19.72 A, giving a unit cell volume of 2530.8 × 10^{-24} cm^3. The density, as measured by flotation in a mixture of methyl iodide and 1-iodobutane is 1.69 g cm^{-3}. Systematic absences, $h00$ when $h = 2n + 1$, $0k0$ when $k = 2n + 1$, $00l$ when $l = 2n + 1$, established the space group as $P2_12_12_1(D_2^4)$. There appear to be eight molecules of $C_{10}H_{14}BrClO_3S$ (M = 329.8) in the unit cell.

Reaction of (+)-3-Bromocamphor-9-sulfonyl Chloride with Caldariomycin. A solution of 53.8 mg of caldariomycin and 249.9 mg of the sulfonyl chloride in 1.5 ml of pyridine stood at room temperature for 48 hr and then excess 2 *N* hydrochloric acid and ether were added. The aqueous layer was removed, and a white crystalline solid (115 mg, 44%, mp 180–182.5°) which separated from the ether solution was filtered. This was recrystallized from 95% ethanol to give white needles of caldariomycin bis[(+)-3-bromocamphor-9-sulfonate], mp 183–184°.

Anal. Calcd for $C_{25}H_{34}Br_2Cl_2O_8S_2$: C, 39.64; H, 4.41; Br, 21.10. Found: C, 39.92; H, 4.57; Br, 20.97.

X-Ray Work on Caldariomycin Bis[(+)-3-bromocamphor-9-sulfonate]. The cell parameters were determined from precession photographs using Mo Kα radiation (λ = 0.7107 A), and the density was determined by flotation in a mixture of carbon tetrachloride, bromoform, and iodobenzene.

Data Collection. We discovered that after approximately 100 hr of exposure at 35 kv and 20 ma the X-ray reflections became diffuse and there was an accompanying loss in over-all intensity of the X-ray diffraction pattern. Within the 100-hr time period, no relative changes in intensity could be discerned. Three different crystals, all mounted about the *c* axis, were used to record the intensity data on equiinclination Weissenberg photographs (Cu Kα radiation). The levels $hk0$ and $hk1$ were obtained from one crystal, the levels $hk2$ and $hk3$ from another, and the levels $hk4$ and $hk5$ from a third. The intensities of 1259 independent structure amplitudes were measured by visual comparison with a series of timed exposures of a typical spot. The intensities were corrected for Lorentz and polarization effects and spot shape variation.[11] No correction was made for absorption.

Structure Determination and Refinement. The section P (u, $1/2$, w) of the three-dimensional Patterson map contained two peaks from which we were able to assign the x and z coordinates of the two bromine atoms in the crystal asymmetric unit. These assignments were consistent with other large peaks considered to arise from vectors between Br atoms not related by symmetry. A Fourier map with phases based on the two bromine atoms revealed the positions of the two chlorine and two sulfur atoms. A second Fourier map allowed the positions of the atoms comprising the caldariomycin ring and one of the camphor groups to be determined, but the second camphor group could not be clearly discerned. At this point, we discovered an indexing error in the $hk4$ and $hk5$ levels which accounted for the lack of definition in the first two electron density maps. A third Fourier map, based on the phases of 27 atoms, revealed the positions of all the atoms, other than hydrogen, in the entire molecule. With all atoms included in a structure factor calculation, the crystallographic R factor was 0.25. Two cycles of full-matrix, least-squares refinement[12] of the positions and isotropic thermal parameters of the two bromine atoms reduced R to 0.23. All reflections were given unit weight and the quantity minimized was $\Sigma w(|F_{obsd}| - |F_{calcd}|)^2$. Two further cycles, refining the positional and isotropic thermal parameters of the two bromine, two chlorine, and two sulfur atoms reduced R to 0.22. Limitation of computer storage capacity prevented full-matrix, least-squares refinement on all atoms. Accordingly, the molecule was divided into two groups of atoms, with the two bromine atoms and the nine atoms of the caldariomycin moiety common to both groups. The groups were refined alternately, and four cycles of such partial refinement varying positional and isotopic temperature factors reduced R to 0.16. Adjustment of the interlevel scale factors, and finally four cycles of refinement, with anisotropic temperature parameters being introduced for the bromine, chlorine, and sulfur atoms gave a final R factor of 0.12 on 1259 independent, nonzero structure amplitudes. Once again the molecule was divided into two groups as described above. A difference map, calculated from the final $F_{obsd} - F_{calcd}$ values, did not indicate significant residual electron density in the unit cell.

The estimated standard deviations (Table I) may be slightly optimistic as the terms containing the interactions between atoms in different groups were set equal to zero when the inverse matrix was calculated. As the final cycle of least squares varied atoms C(11)–C(15), C(1′)–C(10′), O(1′)–O(4′), O(4), S′, Cl(1), Cl(2), Br, and Br′, the estimated standard deviations of the parameters of the bromine and chlorine atoms were obtained from the inverse matrix appropriate to this group. The atomic scattering curves used in the present study were taken from the compilation by Ibers.[13] The bromine curve was corrected for the real component of anomalous dispersion.[14]

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