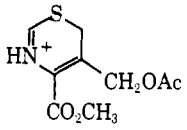
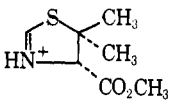
Table I. Isotopic Content of Cephalosporin C and Penicillin N Derivatives



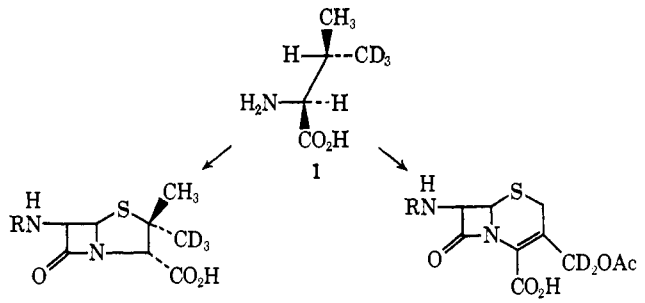
m/e 230



m/e 174

Valine precursor	Ceph C A d_0	d_1	d_2	d_3	d_4	d_5	d_6	Pen N B d_0	d_1	d_2	d_3	d_4	d_5	d_6
(2*S*,3*S*)-[4,4,4-$^2H_3$]-Valine[a]	63	4	30	3	0	0	0	56	0	1	43	0	0	0
(2*S*,3*R*)-[4,4,4-$^2H_3$]-Valine[b]	78	20	2	0	0	0	0	60	1	8	31	0	0	0
(2*RS*)[$^2H_6$]-Valine[c]	72	10	7	10	1	0	0	71	0	0	0	0	0	29

[a] The deuterium contents were 1% d_2 and 99% d_3. [b] 3% d_0, 20% d_2, and 77% d_3. [c] 95% d_6 and 5% d_5.

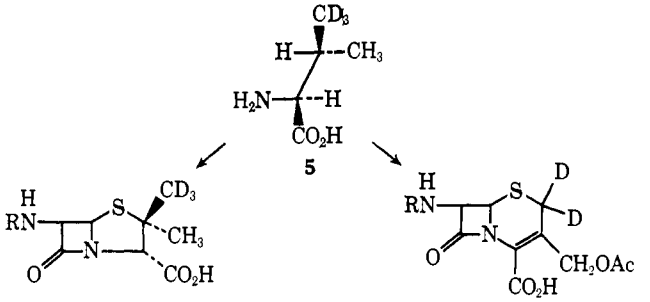


prominent (P + 3) peak at *m/e* 177. With cephalosporin C originating from **1**, an intense (P + 2) peak at *m/e* 232 was evident indicating that two deuterium atoms were retained at the exocyclic C-17 position.

A strong (P + 3) peak was observed with the penicillin N derivative from **5** again demonstrating that all three deuteriums in (2*S*,3*R*)-[4,4,4-$^2H_3$]valine were incorporated intact into the β-methyl group of the penam nucleus. However, mass spectrometric analyses of the cephalosporin C derivative derived from **5** did not give a clear picture as the results showed 77.7% d_0, 20.3% d_1, and 2.0% d_2 in the derivative suggesting that either one or two deuteriums may be incorporated into the endocyclic C-2 position of the cephem nucleus. In an attempt to acquire more accurate isotopic ratios, (3*RS*)-[$^2H_6$]valine (95% d_6) was prepared[13] with the objective of obtaining a cephalosporin C sample devoid of interferences from the natural abundances of (P + 1) and (P + 2) peaks, but, again, the results were inconclusive since low but significant quantities (1%) of (P + 4) and (9.8%) of (P + 3) peaks were noted.

Although one may envisage a biosynthetic pathway whereby the (*R*)-methyl group of L-valine is metabolized to the oxidation state of an aldehyde, which then undergoes subsequent ring closure, it is also possible that this ambiguity may be the result of intermolecular deuterium scrambiling in the mass spectrometer. Thus, the cephalosporin C derived from (2*RS*)-[$^2H_6$]valine was converted into its *N*-benzoyl derivative shifting the C-14 proton resonance signal further downfield and clearly separated from the AB quartet representing the C-2 methylenes at δ 3.42 and 3.69. Careful quantitative pmr[14] analyses of this *N*-benzoylcephalosporin C revealed no differences in the areas of these C-2 protons, suggesting that the endocyclic methylenes at C-2 possessed two deuteriums.

To answer this question more definitively, an incubation with washed cells of *C. acremonium* was carried out in 80% D_2O.[15] The pmr spectrum of the resulting cephalosporin C showed the complete absence of deuterium at the C-2 position as evidenced by the complete symmetry of the AB quartet,[16] thereby establishing that both protons at C-2 originated from the (3*R*)-methyl of L-valine.



These experimental data make it unlikely that the formation of the Δ^3-cephem nucleus proceeds *via* a Δ^2-cephem intermediate. Further, they are in accord with, although they do not prove, the participation of an α,β-dehydrovalinyl derivative in the biosynthesis of the penam nucleus.

Acknowledgment. Grateful acknowledgment is made to the National Institutes of Health for their generous support of this research through Grant AI-10,519. The support of the Medical Research Council and the National Research Development Corporation is also gratefully acknowledged. We thank Professor Howard Whitlock for some of the nmr analyses and Dr. Roger Foltz of Battelle for many of the mass spectrometric analyses of the β-lactam antibiotics.

(13) N. F. Albertson, *J. Amer. Chem. Soc.*, 72, 1396 (1950).

(14) Nuclear magnetic resonance spectra were obtained on a Bruker HX-90E spectrometer. Ten per cent D_2O served as the lock signal, and chemical shifts are given in parts per million relative to tetramethylsilane.

(15) B. C. Carlstedt, H. L. Crespi, M. I. Blake, and J. J. Katz, *J. Pharm. Sci.*, 60, 1661 (1971), reported the failure to label the methyl groups of benzylpenicillin from D_2O during fermentation.

(16) The stereospecific incorporation of deuterium at this carbon during biosynthesis would result in an asymmetric quartet. Also, integrals of the C-2 protons at δ 3.88 and 4.12 revealed no significant differences. Substantial amounts of deuterium were incorporated into the C-7, C-6, the acetoxyl group, and the α-aminoadipic acid side chain of cephalosporin C.

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Use of Copper Hexafluoroacetylacetonate for the Determination of the Absolute Configuration of Alcohols

Sir:

In the following, the methods for correlating the absolute configurations of 1,2- and 1,3-glycols with signs of the CD Cotton effects in the presence of Pr-

Table I. Results from the Addition of $Cu(hfac)_2$ (in CCl_4)

No.	Compound	Predicted chirality	$\Delta\epsilon_{333}$[a]	Concn $Cu(hfac)_2$ (M)	Concn substrate (M)
1	(2*R*)-3-Methyl-2-butanol	(+)	*h*, *i*		
2	(2*R*)-2-Octanol	(+)	*i*		
3	5-Pregnan-20-ol-3-one	(+)	*j*		
4[c]		(−)	−0.5	1.0×10^{-4} [b]	2.6×10^{-3}
5	(−)-Linalool[d]	(−)	*i*		
6	(+)-Neroldiol[d]	(+)	+0.5[g]	1.1×10^{-4}	5.0×10^{-3}
7	(−)-Menthol	(+)	*i*		
8	Lanosterol	(−)	−0.9	1.0×10^{-4}	2.0×10^{-2}
9	(−)-*cis*-Menthen-2-ol	(−)	−1.0[g]	1.1×10^{-4}	7.0×10^{-3}
10	(+)-Cedrol	(−)	*i*		
11[e]		(−)	−0.4	1.0×10^{-4} [b]	5.0×10^{-4}
12	Macarangonol[f]	(−)	−0.1	1.0×10^{-4} [b]	5.0×10^{-4}
13	D-(−)-Pantolactone	(−)	−1.2	1.0×10^{-4}	4.7×10^{-3}
14		(+)	+0.1	1.0×10^{-4}	5.0×10^{-4}
15		(−)	−0.6	1.0×10^{-4}	2.1×10^{-3}

[a] The $\Delta\epsilon$ is based on the concentration of $Cu(hfac)_2$. [b] The concentration of $Cu(hfac)_2$ is approximate since it was added as a solid. [c] Gift of Dr. G. Ellestad, Lederle Laboratories. [d] Gift of Dr. B. J. Kane, Glidden-Durkee. [e] Gift of Professor G. Stork, Columbia University. [f] W. H. Hui, K. K. Ng, N. Fukamiya, M. Koreeda, and K. Nakanishi, *Phytochemistry*, **10**, 1617 (1971). [g] In hexane. [h] See Figure 1. [i] See Figure 3. [j] See Figure 4.

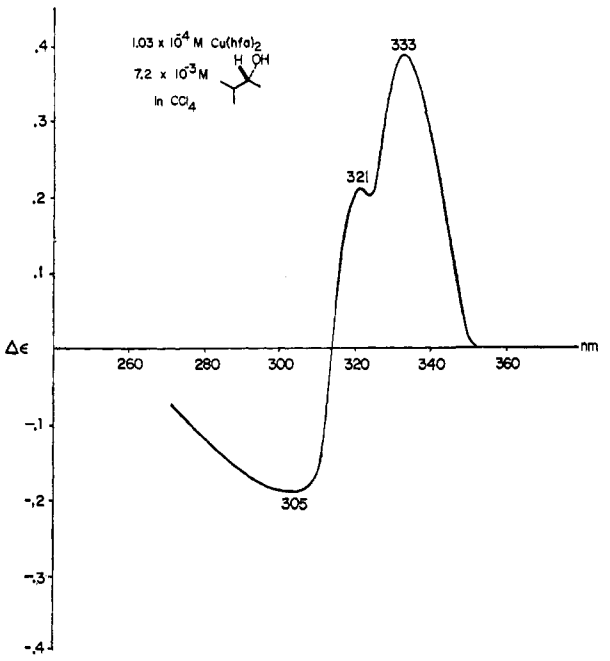
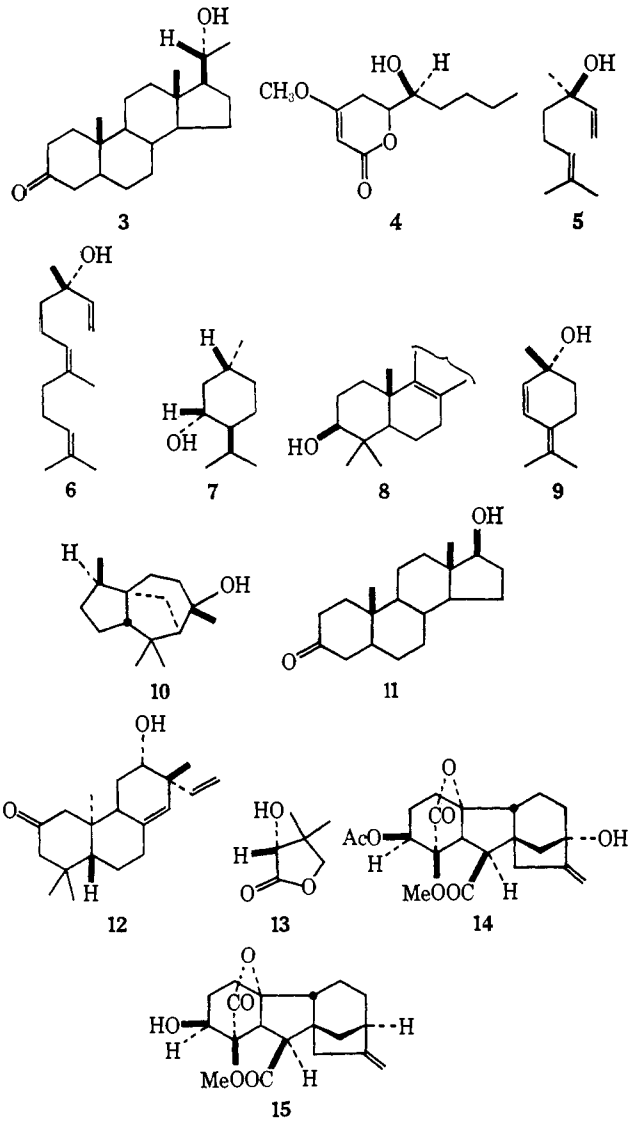


Figure 1. The induced CD resulting from a mixture of 1.03×10^{-4} M $Cu(hfac)_2$ and 7.2×10^{-3} M (−)-3-methyl-1,2-butanol in CCl_4.

$(dpm)_3$[1,2] and $Ni(acac)_2$[2,3] have been extended to alcohols, both cyclic and acyclic.

Although the induced CD's at 315 nm were employed for glycols, it was found that with monofunctional alcohols[4] the small intensities precluded their usage as a structural probe. It was expected that a transition metal with fluorinated ligands would be a much better Lewis acid and hence alcohol acceptor. Indeed when copper hexafluoroacetylacetonate [$Cu(hfac)_2$][5] was added to *ca.* 10^{-3} M solutions of optically active alcohols or amines, it was found that stable and readily measurable CD extremas were obtained at 305–335 nm and that the signs could be used for deducing absolute configurations.[6]



(1) K. Nakanishi and J. Dillon, *J. Amer. Chem. Soc.*, **93**, 4058 (1971).
(2) J. Dillon and K. Nakanishi, *J. Amer. Chem. Soc.*, **96**, 4057 (1974).
(3) J. Dillon and K. Nakanishi, *J. Amer. Chem. Soc.*, **96**, 4059 (1974).
(4) However, amines do give induced CD's with $Pr(dpm)_3$: G. N. Mitchell and F. I. Carroll, *J. Amer. Chem. Soc.*, **95**, 7912 (1973).
(5) For the synthesis of $Cu(hfac)_2 \cdot H_2O$ see J. A. Bertrand and P. I. Kaplan, *Inorg. Chem.*, **5**, 489 (1966). The purple anhydrous complex is obtained after being kept under a vacuum over P_2O_5 for several hours.

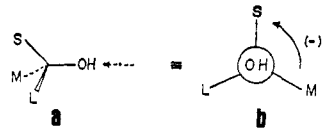


Figure 2. The bulk relationship presented results in a negative Cotton effect at *ca.* 333 nm. Here L, M and S stand for large, medium, and small groups, respectively.

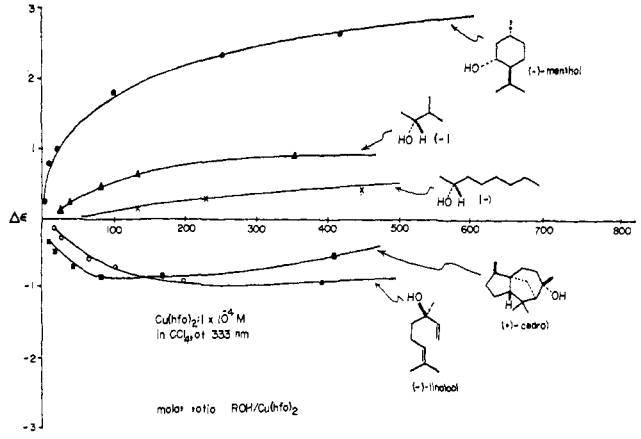


Figure 3. The variation of the $\Delta\epsilon$ at 333 nm for $Cu(hfac)_2$ (1.0×10^{-4} *M*) with increasing concentration of alcohol in CCl_4.

A typical curve is shown in Figure 1. It consists of long wavelength maxima at *ca.* 333 and 321 nm and a short wavelength of opposite sign at *ca.* 300 nm (split CD). This is similar to those found for the $Pr(dpm)_3$, $Ni(acac)_2$, and dibenzoate chirality methods. For purposes of interpretation, only the longest wavelength extrema is chosen. Comparison of the CD data and the known configurations of these compounds leads to the following correlation. A model of the compound in question is observed from the direction of the functional group (Figure 2a). If the bulk relationship[7] between the substituents at the asymmetric carbon is as presented in Figure 2b, *i.e.*, counterclockwise, then a negative Cotton effect will be observed at *ca.* 333 nm.

As has been reported in previous studies, the magnitude of $\Delta\epsilon$ is related to the dryness of solvent and the concentration of optically active species (Figure 3). For these reasons the following procedure is recommended for measurements. A solution 10^{-4} *M* in $Cu(hfac)_2$ and from 10^{-2} to 10^{-3} *M* in alcohol is mixed in dry hexane or CCl_4. The results obtained upon the addition of $Cu(hfac)_2$ to various alcohols are presented in Table I.

The bulk relationships of the substituents around most of the isolated hydroxyl groups follow directly from the drawings. Difficulties arise in compounds such as **10** where nonneighboring carbons play a dominant roll. Here space filling models have to be employed to distinguish the importance of those carbons.

Addition of the Cu reagent to ketones and enone systems produces changes in the CD extrema. This behavior precludes the use of compounds containing enones, since the uv bands are at *ca.* 340 nm and in the same region used in this method. On the other hand unhindered ketones (**3** and **12**) can be used since the CD resulting from the hydroxyl groups falls at a longer wavelength (Figure 4).

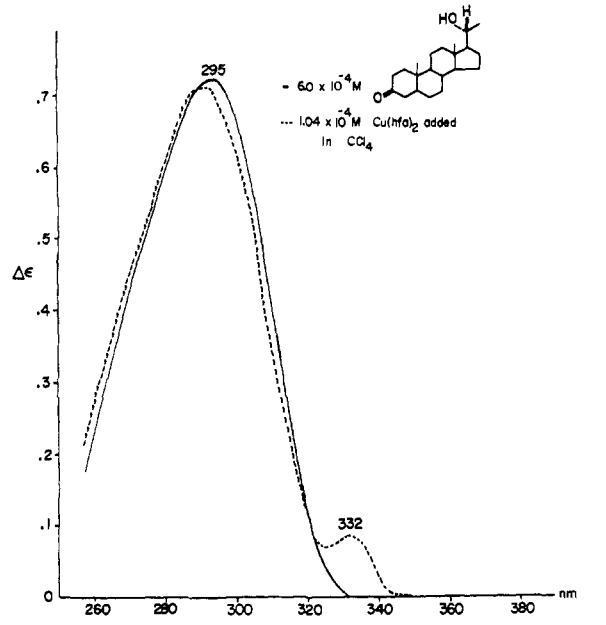


Figure 4. (——) The CD resulting from entry (6.0×10^{-4} *M*). (---) That resulting upon the addition of 1.04×10^{-4} *M* $Cu(hfac)_2$ in CCl_4.

We have not as yet extended this method to amines but the few cases studied gave results similar to those of alcohols; however, the extreme of the amine–$Cu(hfac)_2$ complexes are at *ca.* 310 nm.

Care should be taken in the interpretation of results obtained from compounds containing ketones with, nearby chiral centers[8] and derivatized hydroxyl groups *e.g.*, OAc or OBz, since these also give induced CD's.[9,10]

(6) The strong uv transition permits usage of dilute substrate solutions (*ca.* 10^{-3} *M*). Andersen, *et al.*, have independently developed a similar method employing the d–d transition of $Eu(fod)_3$; however, this method necessitates the usage of more concentrated solutions, 0.04–0.17 *M*. See N. H. Andersen, B. J. Bottino, A. Moore, and J. R. Shaw, *J. Amer. Chem. Soc.*, **96**, 603 (1974).

(7) Polarizabilities of substituents seem to be relatively unimportant; if they were dominating, the predicted results in pantolactone **13** would be opposite to that observed.

(8) Evidently ketones which have a chiral center close to the oxygen, *e.g.*, camphor, give an induced CD from the interaction with $Cu(hfac)_2$; however, this does not occur in cases where the ketone is not close to a bulky center, *e.g.*, cholestan-3-one.

(9) Acetylation of entry **1** results in a 65% reduction of the observed CD.

(10) Supported by NSF GP 40087.

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Use of Complexing Agents for Determining the Absolute Configurations of α-Glycols and α-Amino Alcohols. Basic Systems

Sir:

We previously reported that $Pr(dpm)_3$ can be used for determining the absolute configuration of cyclic α-glycols,[1] and this was extended in a limited way to acyclic sec/tert α-glycols during the course of absolute configurational studies of the insect juvenile hormone.[2] As the measurements are run in organic solvents, the

(1) K. Nakanishi and J. Dillon, *J. Amer. Chem. Soc.*, **93**, 4058 (1971).
(2) K. Nakanishi, D. A. Schooley, M. Koreeda, and J. Dillon, *Chem. Commun.*, 235 (1971).