ficant activity in the fluoride assay system, and the F enzyme has none in the hydroxylamine assay system.

In the absence of hydroxylamine, Reaction 1 can be coupled with Reaction 3 (catalyzed by HIV CoA carboxylase[13]) to yield HMG CoA. The action of the HMG CoA cleavage enzyme[14] converts this product quantitatively to equimolar amounts of acetyl CoA and acetoacetate (Reaction 4). The data in Table II demonstrate that the H enzyme is active in this system whereas the F enzyme is without effect. It is concluded, therefore, that the H enzyme and the carbon dioxide-activating enzyme are probably identical, but that the F enzyme plays no role in carbon dioxide activation for HIV CoA carboxylation. In accord with these findings, the coupling of Reactions 1, 3 and 4 in a heart extract free of pyrophosphatase has been found to furnish approximately equimolar amounts of pyrophosphate and acetoacetate.

TABLE II

CARBON DIOXIDE FIXATION BY THE COUPLED ACTION OF H ENZYME AND β-HYDROXYISOVALERYL CoA CARBOXYLASE

The test system contained 500 μmoles of potassium bicarbonate, 5 μmoles of Versene, 50 μmoles of magnesium chloride, 10 μmoles of glutathione, 20 μmoles of ATP, 2 μmoles of HIV CoA, and, where indicated, H enzyme (2.3 mg. of protein), F enzyme (2.0 mg. of protein), and a pig heart fraction containing HIV CoA carboxylase but free of H and F enzymes (5.2 mg. of protein), in a final volume of 3.0 ml. An excess of HMG CoA cleavage enzyme was present in each experiment. Incubation was for 60 minutes at 38°. HIV CoA was omitted in control experiments.

Enzymes added	μMoles acetoacetate formed[15]
H enzyme + carboxylase	0.60
F enzyme + carboxylase	0
H enzyme	0.07
Carboxylase	0.02

Since the results presented establish two enzymatic steps in the carboxylation of HIV CoA, it seemed of particular interest to determine which of these might be lacking in biotin deficiency.[16] Enzyme extracts prepared from the livers of biotin-deficient rats possess no HIV CoA carboxylase activity, but have the same content of H enzyme, F enzyme, and HMG CoA cleavage enzyme as do those prepared from normal livers.[17] The H enzyme has recently been purified 650-fold from pig heart extracts and found to require the presence of Zn^{++} ions for maximal activity.

[13] B. K. Bachhawat, W. G. Robinson and M. J. Coon, *J. Biol. Chem.*, **219**, 539 (1956).

[14] B. K. Bachhawat, W. G. Robinson and M. J. Coon, *ibid.*, **216**, 727 (1955).

[15] Estimated by a modification of the method of S. S. Barkulis and A. L. Lehninger, *J. Biol. Chem.*, **190**, 339 (1951).

[16] G. W. E. Plaut and H. A. Lardy, *J. Biol. Chem.*, **186**, 705 (1950).

[17] Unpublished experiments carried out by the authors in collaboration with Dr. J. F. Woessner and Dr. Henry A. Lardy.

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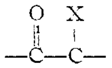
OPTICAL ROTATORY DISPERSION STUDIES. X.[1] DETERMINATION OF ABSOLUTE CONFIGURATION OF α-HALOCYCLOHEXANONES[2]

Sir:

The determination of the absolute configuration of carbonyl compounds by rotatory dispersion measurements[1,3] involves a comparison of the rotatory dispersion curve of the unknown substance with those of reference ketones (or aldehydes) which have been sterically related to D-glyceraldehyde. In connection with an extensive study[4] of the effect of α-substitution upon the rotatory dispersion curves of ketones, there has been made an observation which appears to offer a means of establishing the absolute configuration of cyclohexanones *without requiring a reference compound of known absolute configuration.*

While introduction of an *equatorial* halogen atom (chlorine, bromine but *not* fluorine)[4] in the α-position of a cyclohexanone does not seem to affect the sign of the rotatory dispersion curve of the halogen-free parent ketone,[5] this is not necessarily the case with *axial* bromine or chlorine substituents. In the latter instance, the stereochemistry of the  $-\overset{\overset{\displaystyle O}{\|}}{C}-\overset{\overset{\displaystyle X}{|}}{C}-$ chromophore as a whole becomes dominant and *controls the sign of the Cotton effect.* The gross shape of the dispersion curve can be predicted by examining a model of the appropriate ketone in the following manner:

Place the cyclohexanone ring in such a way that the carbonyl carbon atom is the "head" of the chair and look along the O=C bond as shown by the arrow in I. As demonstrated in Table I, if the axial α-chlorine or bromine atom lies to the *right* (*ca.* 105° angle[7]) of the observer (I, X = Cl or Br), *the single Cotton effect will be positive;* if

TABLE I

Axial α-haloketone	Cotton effect Halogen-free ketone	 α-Haloketone
3α-Bromoandrostan-2-one-17β-ol propionate	Positive	Positive
7α-Bromocholestane-$3\beta,5\alpha$-diol-6-one 3-acetate	Negative	Positive
7α-Bromocholestane-$3\beta,5\alpha$-diol-6-one 3,5-diacetate	Negative	Positive
6β-Bromocholestan-3β-ol-7-one acetate	Negative	Positive
9α-Bromoergostan-3β-ol-11-one acetate	Positive	Positive
12α-Bromoergostan-3β-ol-11-one acetate	Positive	Negative
12α-Chloro-11-ketotigogenin acetate	Positive	Negative
12α,23-Dibromo-11-ketotigogenin acetate	Positive	Negative
Methyl 11β-bromo-3α-acetoxy-12-ketocholanate	Positive	Negative
2α-Bromofriedelin	Negative	Negative
4α-Bromofriedelin	Negative	Positive

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[1] Paper IX, C. Djerassi and W. Klyne, *Chemistry and Industry*, 988 (1956).

[2] Supported by Grant No. CY-2919 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

[3] C. Djerassi, R. Riniker and B. Riniker, THIS JOURNAL, **78**, 6362 (1956).

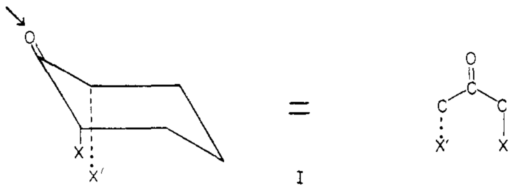
[4] C. Djerassi, *et al.*, to be published. The rotatory dispersion curves of the ketones listed in Table I will be reproduced in that article.

[5] For example, cholestan-3-one, 2α-bromocholestanone and 4α-bromocholestanone all exhibit a positive single Cotton effect curve.[6]

[6] For nomenclature in rotatory dispersion work see C. Djerassi and W. Klyne, *Proc. Chem. Soc.*, 55 (1957).

[7] R. C. Cookson, *J. Chem. Soc.*, 181 (1954).

the chlorine or bromine is to the *left* (I, X′ = Cl or Br) the effect will be *negative*.[8]



I

Since the axially-oriented bromine or chlorine substituent in α-halocyclohexanones is often the more stable one,[9] the presently described method for the determination of absolute configuration of such ketones should be rather widely applicable. The steps to be taken are simple and particularly if the first one can be omitted, the entire sequence can be carried out on a micro-scale without using up any material: (a) if both α and α′ positions can be substituted and the exact location of the halogen atom does not follow from its mode of preparation (*e.g.*, epoxide opening followed by oxidation), then this must be established chemically—usually by dehydrohalogenation; (b) the axial orientation[10] of the α-halocyclohexanone must be confirmed by ultraviolet[7] and infrared[9,11] measurements; (c) the rotatory dispersion curve must be measured. It is then possible to determine in the manner illustrated above (*cf.* I) which one of the two absolute configurations leads to the observed (positive or negative) Cotton effect.

Attention should be called to two further applications of rotatory dispersion measurements among α-halocyclohexanones. If the absolute configuration of the parent ketone is known but the location (α or α′) of the axial halogen atom is not, then the rotatory dispersion curve will provide the answer. Conversely, if information concerning the absolute configuration of the ketone and the location of the halogen substituent is available, but spectroscopic determination of the orientation of the halogen atom is obscured by additional carbonyl groups, a decision may be reached by means of the rotatory dispersion curve. An interesting example is provided by the two epimeric methyl 3α-acetoxy-11-bromo-12-ketocholanates[12] which possess virtually identical rotations ($[\gamma]_D$ +44° and +40°) at the conventional sodium D line. However, the rotatory dispersion curves[4] disclose that once rotation measurements are continued in the ultraviolet range, a marked change is noted. In agreement with the postulates made in this paper, the equatorial 11α-bromo isomer exhibits a positive Cotton effect (peak[6] at 295 mμ, [α] +498°) as does the halogen-free 11-ketone,[13] while the axial 11β-isomer shows a strong negative Cotton effect (trough[6] at 337.5 mμ, [α] −1020°).

[8] This effect may be so pronounced that it may carry over into the visible range of the spectrum. Consequently, at times even comparisons of molecular rotation differences (based only on $[\alpha]_D$ values) with steroid reference compounds may be used as has been done in the friedelin series (E. J. Corey and J. J. Ursprung, THIS JOURNAL, **78**, 5041 (1956)).

[9] E. J. Corey and H. J. Burke, *ibid.*, **77**, 5418 (1955), and earlier papers. See, however, W. D. Kumler and A. C. Huitric, *ibid.*, **78**, 3369 (1956).

[10] It appears (ref. 4) that even if the equatorial orientation is the more stable one it might still be possible to use this method if the corresponding *gem*-dihalo ketone is available since one of the halogen atoms must now be axial.

[11] R. N. Jones, D. A. Ramsay, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2828 (1952).

[12] Kindly provided by Dr. T. F. Gallagher, Sloan-Kettering Institute for Cancer Research.

[13] C. Djerassi and W. Closson, THIS JOURNAL, **78**, 3761 (1956).

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MULTILAYER MEMBRANE ELECTRODES[1]

Sir:

We have prepared a new membrane which is permeable almost exclusively to certain ions in the presence of other ions of the same charge. These membranes are multilayers, where transport of the potential-determining ion is *transverse* to the axis of orientation.

Membranes were constructed by cracking a standard slide in half, so as to insure a nearly perfect fit, because the effective membrane pore width was 2.5×10^{-5} cm. Each glass half-slide was coated with 50 Y-type monolayers of barium stearate, the two halves fitted together and cemented in place between slides having holes in their centers, arms were attached for solutions and the potential measured between saturated calomel electrodes.

The resistance of this membrane was 5.8×10^7 ohms. Accordingly, a highly sensitive Keithley Model 200 B vacuum-tube voltmeter accurate to ± 1% was used with proper shielding. A constant asymmetry potential of 1–3 mv. for identical barium chloride solutions was read; constant and stable potential readings were obtained rapidly.

Preliminary results obtained with this Multilayer Membrane Electrode are given. Experimentally determined potentials were corrected for asymmetry. Calculated e.m.f. values were obtained using the tabulated data of Conway[2]; at higher concentrations, mean activity coefficients were used. For dilute ($< 0.02\ M$) solution pairs whose concentration ratio was less than 2:1, calculated e.m.f. values were reasonably accurate.

Multilayer Membrane Electrodes are shown to be reversible to barium ions, even in the presence of high concentrations of sodium ions. A real measure of this specificity cannot be obtained from potential measurements, but is being determined using transport, exchange, and other methods.

The ability of Multilayer Membrane Electrodes to concentrate a specific ionic species without completely immobilizing that species is responsible for its specific function. The glass electrode is an example. This ideal or nearly ideal degree of specificity results from its highly oriented or semi-crystalline state. Pores, in the usual sense, are not present. Transport probably takes place by exchange between adjacent barium ions. Multilayer Membrane Electrodes specific for other cat-

[1] We wish to thank the Office of Naval Research and the U. S. Public Health Service for the support given this work.

[2] B. E. Conway, "Electrochemical Data," Elsevier Publishing Co., New York, N. Y., 1952, p. 102.