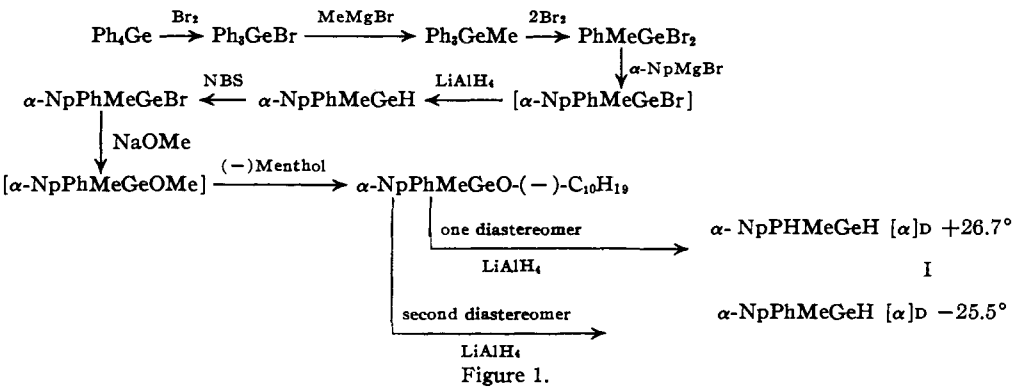

Figure 1.

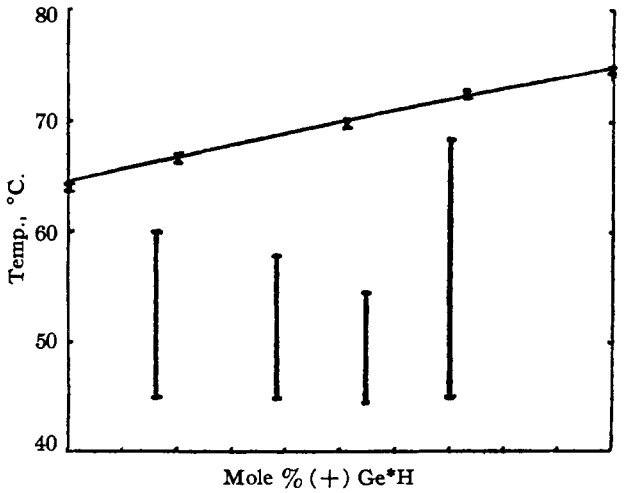

Fig. 2.—Mixture melting point diagram: upper line, (+)Ge*H with (+)Si*H; lower ranges, (+)Ge*H with (−)Si*H.

the same, regardless of whether the central atom is silicon or germanium, these results seem surprising and are being investigated.

An investigation of the stereochemistry of reactions of methyl-α-naphthylphenylgermane is now being undertaken in order to compare the results with those of its silicon analog. Preliminary evidence indicates that bromination of the germane involves complete racemization under a variety of conditions, whereas the silicon analog is found to brominate stereospecifically with retention of configuration[13] under the same conditions.

Treatment of triphenylbromogermane[7] in refluxing xylene with methylmagnesium iodide gave an almost quantitative yield of methyltriphenylgermane, m.p. 66–67°. Bromination with 2 mole equivalents of bromine in ethylene dibromide at 95° for 17 hr. gave 89% of methylphenyldibromogermane, b.p. 139–140° (15 mm.), $n^{25}D$ 1.5962, which was treated with one equivalent of α-naphthylmagnesium bromide in ether–benzene. On consumption of the Grignard reagent (negative Gilman color test) the entire reaction contents were added to excess lithium aluminum hydride in ether. Work-up gave 70% of methyl-α-naphthylphenylgermane, b.p. 155° (0.5 mm.), m.p. 50–51°. Bromination with one equivalent of N-bromosuccinimide in refluxing carbon tetrachloride gave 82% of methyl-α-naphthylphenylbromogermane, m.p. 58–60°.

Treatment of methyl-α-naphthylphenylbromogermane with slightly more than one equivalent of sodium methoxide in excess methanol, followed by replacement of the methanol with xylene by distillation, and then addition of 1.4 equivalents of (−)menthol, followed by azeotropic distillation of methanol over 12 hr., gave on distillation 90% of sirupy methyl-α-naphthylphenyl-(−)-menthoxygermane, b.p. 195–196° (0.2 mm.). This material was dissolved in two volumes of pentane and cooled in an ice bath to give, as a first crop, 34% of methyl-α-naphthylphenyl-(−)-menthoxygermane, m.p. 86–91°, $[\alpha]^{22}D$ −49.3° (*c* 10.1, cyclohexane). Reduction of this material with lithium aluminum hydride in ether over 6 hr. gave in two crops 96% of (+)-methyl-α-naphthylphenylgermane, which after recrystallization from ethanol had m.p. 74–75°, $[\alpha]^{25}D$ +26.7° (*c* 10.6, cyclohexane).

Anal. Calcd. for $C_{17}H_{16}Ge$: C, 69.71; H, 5.51. Found: C, 69.23; H, 5.56.

After removal of additional crops of solid menthoxy compound totalling 59%, the residual oil, $[\alpha]^{20}D$ −59.6° (*c* 8.8, cyclohexane), was similarly reduced with lithium aluminum hydride to give 85% of (−)-methyl-α-naphthylphenylgermane, m.p. 74–75°, $[\alpha]^{25}D$ −25.5° (*c* 11.3, cyclohexane).

Bromination of (+)-methyl-α-naphthylphenylgermane, using either N-bromosuccinimide in carbon tetrachloride at 0° or bromine in carbon tetrachloride at 0°, gave methyl-α-naphthylphenylbromogermane with no observable optical activity.

Weighed samples of the appropriate silanes and germanes were dissolved in ether, and the ether then was removed under reduced pressure. The melting points of the resulting solids were then determined (Fig. 2). Analyses and infrared spectra were in agreement with the assigned structures of all compounds.

Acknowledgments.—Part of this research was supported by the National Research Council of Canada. Grateful acknowledgment is made to the Germanium Information Centre for a gift of germanium tetrachloride.

(14) Cities Service Corporation Fellow, 1962–1963.

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RECEIVED MAY 1, 1963

Optical Rotatory Dispersion Studies. LXXXIV.[1] Studies of Conformational Mobility by Low-Temperature Circular Dichroism Measurements[2]

Sir:

In a recent paper[3] we pointed out the frequent interchangeable use of the phenomenologically closely related physical methods, optical rotatory dispersion (O.R.D.) and circular dichroism (C.D.), in various stereochemical problems. Furthermore, it was emphasized[3,4] that, in certain instances, either O.R.D. or

(1) Paper LXXXIII: C. Djerassi, H. Wolf, D. A. Lightner, E. Bunnenberg, K. Takeda, T. Komeno and K. Kuriyama, *Tetrahedron*, in press.

(2) Supported by the National Science Foundation (Grant No. G-19905).

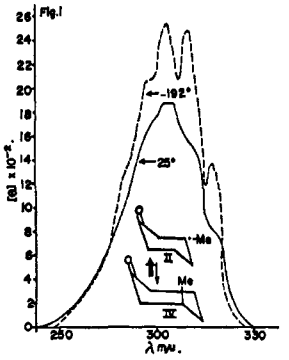
(3) C. Djerassi, H. Wolf and E. Bunnenberg, *J. Am. Chem. Soc.*, **84**, 4552 (1962).

(4) (a) C. Djerassi, H. Wolf and E. Bunnenberg, *ibid.*, **85**, 324 (1963); (b) K. Mislow, E. Bunnenberg, R. Records, K. Wellman and C. Djerassi, *ibid.*, **85**, 1342 (1963).

C.D. will offer information not readily available from either one alone and, in such cases, the combined use of both physical tools is indicated. We now wish to record some applications of C.D. which offer great promise in the study of conformational analysis.

Low-temperature O.R.D. or C.D. measurements as yet have not been recorded in the literature, although they might be expected to shed much light on problems of conformational mobility because of the great sensitivity of these two parameters[5] to conformational changes. We now have constructed a cell[6] which permits ready measurements of C.D. over a wide temperature range. In particular, measurements close to the boiling point of liquid nitrogen are performed routinely. While an expected bonus of such low-temperature work is the greatly increased vibrational structure[7] (see Fig. 1–4)—of potential use for "fingerprinting" purposes—the most important application lies in the area of conformational studies. We shall cover this subject *in extenso* in our detailed articles dealing with the exploitation of such low-temperature measurements and we now wish only to illustrate some of our results in the field of optically active ketones. All of the present measurements were conducted in EPA solvent (5 vol. ether:5 vol. isopentane:2 vol. ethanol) and corrected for volume contraction.

(+)-3-*t*-Butylcyclohexanone (I)[8] does not show any substantial change in the C.D. curves measured at 25° or at −192°, the reduced rotational strength,[9] $[R]$ being +2.18 at 25° and +2.32 at liquid nitrogen temperature. (+)-3-Methylcyclohexanone (II), however, shows a considerable increase (Fig. 1) in rotational strength ($[R]^{25°}$ +1.78 *vs.* $[R]^{-192°}$ +2.34) on going to lower temperature, which can be rationalized with the existence of a small amount of the axial conformer IV and/or a twist form at room temperature, which is known[10] to make a negative rotatory contribution and which is essentially "frozen out" at −192°. In the 3-*t*-butyl analog I, such conformational mobility is expected to be minimal[11] even at room temperature. Particularly striking is the situation with (+)-*trans*-2-chloro-5-methyl-cyclohexanone, where the existence of a conformational equilibrium between the diequatorial form III and the diaxial conformer V had been demonstrated earlier[12] by rotatory dispersion measurements in solvents of different polarity. Not only does conformer III exhibit a positive and V a negative Cotton effect, but the position of the respective extrema differs by nearly 25 mμ. The presence of the two conformers III and V can also be demonstrated (Fig. 2) by keeping the solvent constant (EPA) and scanning the C.D. curve over a wide temperature range. The two extremes (25 and −192°) are reproduced in Fig. 2 and it will be noted that the negative C.D. curve centered at 325 mμ and corresponding to the diaxial conformer V disappears as the temperature is lowered. Conversely, the area under the positive C.D. curve (positive C.D. maximum[9b] at 288 mμ) increases greatly at lower temperature due to the increased preponderance of the diequatorial form III. Reproduced in Fig. 2 are the room temperature C.D. curves of *trans*-2-chloro-5-methylcyclohexanone in two non-polar solvents, carbon tetrachloride and isoöctane, where increased amounts of the axial conformer V are found.[12] The rotational strength of pure V is expected to be so much greater[10,12] than that of III that, in isoöctane solution, the contribution of the equatorial form III cannot be detected (Fig. 2) through a positive C.D. curve and is indicated only by the shoulder on the low-wave length end of the negative curve.



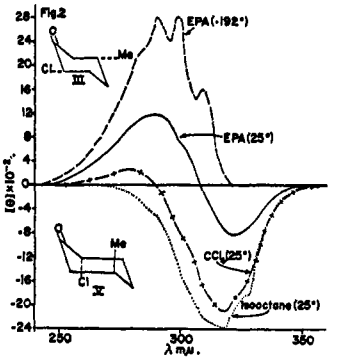
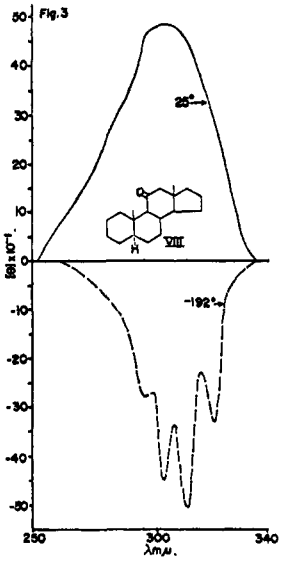


Fig. 1.—Circular dichroism curves (EPA solvent) of (+)-3-methylcyclohexanone (II) at 25° and −192°.

Fig. 2.—Circular dichroism curves of (+)-*trans*-2-chloro-5-methylcyclohexanone (III ⇄ V) in carbon tetrachloride (25°), isoöctane (25°) and EPA solvent (25° and −192°).



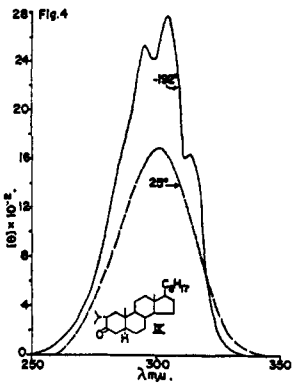


Fig. 3.—Circular dichroism curves (EPA solvent) of 5α-androstan-11-one (VIII) at 25° and −192°.

Fig. 4.—Circular dichroism curves (EPA solvent) of 2α-isopropylcholestan-3-one (IX) at 25° and −192°.

Two other pertinent illustrations of conformational mobility uncovered by low-temperature C.D. studies are the following. The reduced rotational strength of the fused cyclopentanone 5α-androstan-16-one (VII) is essentially unchanged over a wide temperature range ($[R]^{25°}$ +18.7 *vs.* $[R]^{-192°}$ +18.1), while that of (+)-3-methylcyclopentanone (VI) shows a marked augmentation upon lowering of the temperature ($[R]^{25°}$ +6.02 *vs.* $[R]^{-192°}$ +9.88), indicative of a conformational equilibrium at room temperature. Even more

(5) For general survey, see C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., New York, N. Y., 1960; for most recent paper and other leading references, see C. Beard, C. Djerassi, J. Sicher, F. Sipos and M. Tichy, *Tetrahedron*, in press.

(6) The particulars will be described in one of our detailed articles, but we wish at this time to acknowledge the assistance of Mr. Frank Pool of our machine shop.

(7) This was to be expected since ultraviolet absorption spectra at low temperatures are known to exhibit increased resolution; for pertinent references, see R. L. Sinsheimer, J. F. Scott and J. R. Loofbourow, *J. Biol. Chem.*, **187**, 299 (1950).

(8) C. Djerassi, E. Warawa, R. E. Wolff and E. J. Eisenbraun, *J. Org. Chem.*, **25**, 917 (1960).

(9) (a) For definition and calculation from C.D. curve, see A. Moscowitz, Chapter 12 in ref. 5, especially p. 170; (b) for C.D. nomenclature see C. Djerassi and E. Bunnenberg, *Proc. Chem. Soc.*, in press.

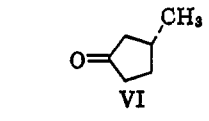
(10) W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne and C. Djerassi, *J. Am. Chem. Soc.*, **83**, 4013 (1961).

(11) See S. Winstein and N. J. Holness, *ibid.*, **77**, 5562 (1955).

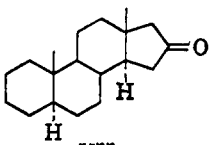
(12) C. Djerassi, L. E. Geller and E. J. Eisenbraun, *J. Org. Chem.*, **25**, 1 (1960); J. Allinger, N. L. Allinger, L. E. Geller and C. Djerassi, *ibid.*, **26**, 3521 (1961)

striking and unexpected is the situation illustrated in Fig. 3 with 5α-androstan-11-one (VIII), where the low temperature C.D. measurement did not only uncover the anticipated vibrational structure, but also resulted in an inversion of the C.D. curve from weakly positive (room temperature, in accord with earlier[13] O.R.D. results) to negative (liquid nitrogen). We interpret these results in terms of the existence of another, hitherto unsuspected, conformer of ring A at room temperature, the tendency toward departure from the standard all-chair form presumably being due to the interference between the equatorial C-1 hydrogen and the 11-keto function.

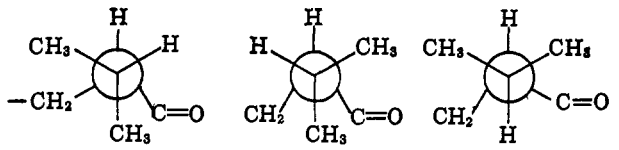
Finally, we cite in Fig. 4 an application of low temperature C.D. measurements to studies of free-rotational isomerism. While the rotational strengths of cholestan-3-one or its 2α-methyl homolog do not differ at −192° and 25°, a considerable increase is observed (Fig. 4) at lower temperature in 2α-isopropylcholestan-3-one (IX).[14] Of the three most obvious rotamers around the C-2 bond, according to the octant rule[10] IXB would be expected to have the least effect (as compared to cholestan-3-one), while IXC would yield a more negative and IXA a more positive rotatory contribution. Evidently at lower temperatures, conformer IXA is the preferred one, which seems reasonable since it does not exhibit the eclipsing between one of the methyl groups and the carbonyl function present in IXB and IXC.



VI



VII



IXa IXb IXc

In addition to the many qualitative applications in stereochemistry which are opened up by such low temperature measurements, we are also investigating some of the more interesting quantitative aspects. Thus, C.D. studies at several temperatures over the −192° to room temperature range offer a means of determining the energy differences between two conformers or rotamers and details of such work conducted in collaboration with Prof. A. Moscowitz of the University of Minnesota will be described in a forthcoming paper.

(13) E. W. Foltz, A. E. Lippman and C. Djerassi, *J. Am. Chem. Soc.*, **77**, 4359 (1955); C. Djerassi and W. Klyne, *J. Chem. Soc.*, 2390 (1963).

(14) P. A. Hart, unpublished observation from this Laboratory.

(15) National Institutes of Health Postdoctoral Research Fellow, 1962–1963.

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RECEIVED MAY 6, 1963

Some Observations Relative to the Mechanism of Chymotrypsin

Sir:

The inactivation of α-chymotrypsin by diphenylcarbamyl chloride (I), a reagent of high specificity, has recently been described.[1] The following are some of the characteristics of this reaction: (1) Inactivation resulted from a reaction of equimolar stoichiometry. (2) Inactivation could be competitively inhibited by indole. (3) The inactivation of chymotrypsin was approximately one hundred times faster than that of trypsin. (4) Reactivation was rapid and complete in the presence of nucleophilic agents such as hydroxylamine and isonitrosoacetone but occurred at a negligible rate in their absence. (5) No reaction occurred between I and chymotrypsinogen or diethylphosphorylchymotrypsin.

These characteristics, as well as analogy with the reaction of carbamates with acetylcholinesterase,[2] are consistent with the conclusion that I is a new type of specific substrate of chymotrypsin capable of participating in the acylation step of the catalytic mechanism but leading to a relatively stable acyl enzyme intermediate.[3] I, therefore, should be a valuable tool for the study of the acylation step of the mechanism by which specific substrates are hydrolyzed by chymotrypsin. This communication reports the results of such a study.

In order to gain an insight into the number and types of functional groups participating in the acylation mechanism, the effect of pH upon the inactivation process was investigated. The rate of inactivation was determined by measurement of residual enzyme activity as a function of time.[4] Complete inactivation could be obtained throughout the pH range 5.0 to 9.5 with a rate maximum at about pH 7.5 (see Fig. 1). K_i (= K_m) remained constant ($0.6 \pm 0.1 \times 10^{-4}$ M) up to pH 8.2.

The stoichiometry of the inactivation process calls for the release of one mole of H^+ for the formation of each mole of diphenylcarbamyl (DPC) chymotrypsin. Titration studies, however, led to the findings shown in Fig. 2.[5] Close to a theoretical quantity of H^+ could be detected only at pH 5–5.5. Thereafter the amount of H^+ was consistently below the expected value.[6] As can be seen in Fig. 2, different conditions of ionic strength and temperature resulted in a shift but no marked change in the shape of the curve.

The low values between pH 5.5 and 7.5 agree with the findings of Guttreund and Sturtevant[7] and are consistent with their suggestion that acylation of the enzyme resulted in a change in the apparent pK_a of a functional

(1) B. F. Erlanger and W. Cohen, *J. Am. Chem. Soc.*, **85**, 348 (1963).

(2) *Cf.* I. B. Wilson, M. A. Harrison and S. Ginsburg, *J. Biol. Chem.*, **236**, 1498 (1961).

(3) Despite the fact that it causes inactivation, I is a substrate of chymotrypsin because, like conventional substrates, its hydrolysis is mediated by the enzyme *via* a multi-step mechanism involving acylation of chymotrypsin followed by deacylation [*cf.* B. Zerner and M. L. Bender, *J. Am. Chem. Soc.*, **85**, 356 (1963)]. The deacylation step, however, proceeds very slowly. It should be considered to be a *specific* substrate because its rate of reaction with chymotrypsin is almost two orders of magnitude faster than its rate of reaction with trypsin and, as reported in this paper, it has a rather respectable K_m (or K_i depending upon one's point of view).

(4) The methods used were essentially the same as described in ref. 1.

(5) The preparation of α-chymotrypsin (Worthington 3× crystallized, lot no. CDI 6032) was found to be 88% pure (mol. wt. 25,000) by titration with I as well as by a method that utilized the specific chromogenic inactivator 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate (B. F. Erlanger and F. Edel, in preparation). The reaction was carried out under nitrogen in a Radiometer pH-Stat using 0.0087 N NaOH standardized against a National Bureau of Standards preparation of benzoic acid. The diphenylcarbamylchloride used was from Distillation Products, Inc., and recrystallized three times from ethanol to constant m.p. 85° [H. Erdmann and P. Huth, *J. prakt. Chem.* (II), **56**, 7 (1897) report 85°]. It was stable in acetone and isopropyl alcohol and could be recovered pure (by melting point) after distillation of the solvent. Early experiments utilized dimethyl sulfoxide as a solvent for I. Use of this solvent was discontinued when it was found that considerable decomposition of I took place in 30 min. at room temperature. After each run, the apparatus and standard base were checked by the titration of a convenient quantity of standardized hydrochloric acid.

(6) That the low values were not due to denaturation of the enzyme was established by simultaneous assay with the chromogenic inactivator described in ref. 5.

(7) H. Gutfreund and J. M. Sturtevant, *Proc. Natl. Acad. Sci. U.S.*, **42**, 719 (1956).