

liquid SO₂ was distilled in; Ib (0.203 g., 0.0010 moles) was distilled into the mixture, which was cooled in a liquid nitrogen bath. The U-tube was sealed closed and warmed to -78° with vigorous shaking. The solution was filtered at -60° into the n.m.r. tube which was then fused off. The n.m.r. spectrum was determined at -60°; decomposition set in at temperatures above -30°. The spectrum consisted (from lower to higher fields) of three singlets of relative intensity 6.2:3.1:5.7 spaced 0.2 and 0.7 p.p.m. apart. Calibration by replacement of the sample tube with one containing TMS in SO₂ showed the absolute peak positions to be *ca.* τ 7.5, 7.8, 8.5. Relative to internal tropylium hexafluoroantimonate at τ 0.67, the peaks appear at τ 7.47, 7.68, and 8.39.

General Method for the Determination of the Ultraviolet Spectra of Ia, b, c, and d.—The methylene chloride was purified as follows. It was washed with concentrated H₂SO₄, neutralized with aqueous ammonia, washed with water, and dried over CaCl₂ and then KOH. It was refluxed over AlCl₃ overnight and distilled immediately prior to use. The carbonium ions were prepared in methylene chloride solution in the way described above for the preparation of the n.m.r. samples. The ultraviolet spectra were determined in 0.1-mm. and 1.0-cm. quartz cells.

Quenching of Carbonium Ion Ia with Tetramethylammonium Chloride.—To a stirred suspension of 1.34 g. (0.010 mole) of AlCl₃ in 5 ml. of CH₂Cl₂ at -78°, 1.79 g. (0.010 mole) of IId was rapidly added. After warming to room temperature, the solution was syringed dropwise into a stirred solution of 2.2 g. (0.020 mole) of tetramethylammonium chloride in *ca.* 30 ml. of liquid SO₂ at -78°. The SO₂ was allowed to boil away. Pentane was added to the residue and the mixture filtered. The filtrate on evaporation left 1.49 g. of a yellow solid, m.p. 51–53°, which sublimed (45° at 15 mm.) to give 1.43 g. (80%) of IId, m.p. 56–57° (lit.⁷ m.p. 57°), identical (mixture m.p., infrared, n.m.r.) with a known sample.

Quenching of Ib.—Compound Ib was prepared in 3 ml. of methylene chloride from 0.134 g. (0.0010 mole) of AlCl₃ and 0.203 g. (0.0010 mole) of Ib. The methylene chloride was evap-

oratively distilled at 10⁻⁶ mm. and the residue was then pumped on at 10⁻⁶ mm. for 30 min. Sulfur dioxide (3 ml.) was subsequently distilled in at -78°. From a side arm attached to the reaction vessel 0.22 g. (0.002 mole) of tetramethylammonium chloride was rapidly added to the stirred SO₂ solution at -78°. The solution was warmed to -30° and most of the SO₂ was removed by evaporative distillation at 10⁻⁶ mm. The very small amount of liquid remaining was evaporatively distilled at room temperature into another flask, which was then allowed to stand at room temperature and atmospheric pressure to permit the last traces of SO₂ to escape. To ensure complete removal of the SO₂, the remaining liquid was warmed to *ca.* 50° for 1 min. Subsequent evaporative distillation of the liquid at 10⁻⁶ mm. afforded 0.091 g. (57%) of pure (infrared, n.m.r.) 1,2,3,4,4-pentamethyl-3-chlorocyclobutene (IIa).

Quenching of Ic.—The procedure was the same as for Ib. The carbonium ion (0.0010 mole) was prepared in methylene chloride and quenched with 0.22 g. (0.002 mole) of tetramethylammonium chloride to yield 0.096 g. (66%) of pure (infrared, n.m.r.) *cis*- and *trans*-IIc.

Treatment of the Cyclobutenyl Halides with SnCl₄ and ZnCl₂.—The n.m.r. samples were prepared in methylene chloride using the same techniques employed for preparing the n.m.r. samples of the carbonium ions using AlCl₃. Only in the case of IIc was it necessary to store the reaction mixture at -78°. The other mixtures could be warmed to room temperature.

Recovery of IId from a Mixture of IId and SnCl₄.—The technique was the same as for quenching Ia. A mixture of 0.895 g. (0.005 mole) of IId and 0.650 g. (0.025 mole) of SnCl₄ in 5 ml. of methylene chloride was added to 1.1 g. (0.010 mole) of tetramethylammonium chloride in 25 ml. of liquid SO₂ at -78°. Pure IId (m.p., mixture m.p., infrared, n.m.r.) was obtained, after sublimation, in 84% yield.

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Molecular Rotations of Methylcyclohexanols in Relation to Their Structures

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The PM method, a modification of the Kirkwood method¹ of computing optical rotatory power, is shown to be applicable to the methylcyclohexanols.

In 1956, Whiffen² studied the molecular rotation of optically active substances such as derivatives of cyclohexane and tetrahydropyrene, using monochromatic (sodium D line) plane polarized light, and concluded that the predominant optical effect was contributed by the adjacent substituents of the chair form of these molecules. Indeed, he proposed a new method of computing optical molecular rotation. It should be noted, however, that Whiffen's method is only a first approximation; that is, in some cases the smaller rotation effects of more widely separated substituents account for the major part of the optical activity.

According to Kirkwood,¹ the rotation is caused by the dynamic coupling effect between any two members of unit groups in the molecule. Studies³ by the author suggest that Kirkwood's theory must be modified for carbohydrates. This modified method of calculating

molecular rotation is called the PM method⁴ and has been used successfully for carbohydrates,^{5–7} polyhydroxycyclohexanes,⁸ and menthol-like compounds.⁹ This article is a study of the applicability of the PM method to methylcyclohexanols.

The basic relationship of the PM method is

$$[M]_{D^{20}} \equiv \sum_{i \neq k} [\mu]_{D^{20}}^{\text{obs}(ik)} \quad (1)$$

But $[\mu]_{D^{20}}^{\text{obs}(ik)}$ is the partial molecular rotation contributed by the dynamic coupling effect between the groups *i* and *k*, and therefore

$$[\mu]_{D^{20}}^{\text{obs}(ik)} = \zeta_i \zeta_k [\mu]_{D^{20}}^{\text{cal}(ik)} \quad (2)$$

In this equation ζ_i and ζ_k are characteristics of *i* and *k*,

(4) Reference *17 in the previous paper.⁵

(5) S. Yamana, *Bull. Chem. Soc. Japan*, **31**, 558 (1958).

(6) S. Yamana, *ibid.*, **30**, 207, 920 (1957); **31**, 564 (1958); **35**, 1269, 1421, 1950 (1962); **36**, 473 (1963).

(7) S. Yamana, *ibid.*, **30**, 916 (1957).

(8) S. Yamana, *ibid.*, **33**, 1741 (1960); **34**, 1212 (1961).

(9) S. Yamana, *ibid.*, **34**, 1414 (1961).

(1) W. W. Wood, W. Fickett, and J. G. Kirkwood, *J. Chem. Phys.*, **20**, 561 (1952).

(2) D. H. Whiffen, *Chem. Ind. (London)*, 964 (1956).

(3) S. Yamana, *Bull. Chem. Soc. Japan*, **30**, 203 (1957).

TABLE I

Cyclohexanol	Conformation	Unit group ¹²	$\Sigma[\mu]_{D^{20}}^{obs}$ ¹⁴	$[M]_{D^{20}}$ ¹⁴	Ref.
(-)- <i>trans</i> -2-Methyl-	C1	[(OH) ^{1β} , (CH ₃) ^{2α}]	-43.9° (-43.9°)	-43.9°	16
(-)- <i>trans</i> -3-Methyl-	C1	[(OH) ^{1β} , (CH ₃) ^{3α}]	-7.3° (-12.2°)	-8.4°	17
	1C	[(OH) ^{1β} , (CH ₃) ^{3α}]	-7.0° (-11.0°)		
(+)- <i>cis</i> -3-Methyl-	C1	[(OH) ^{1β} , (CH ₃) ^{2β}]	0.0° (0.0°)	2.2°	18
(-)- <i>cis</i> -2-Methyl-	C1	[(OH) ^{1β} , (CH ₃) ^{2β}]	43.9° (43.9°)	-15.3°	16
	1C	[(OH) ^{1β} , (CH ₃) ^{2β}]	-43.9° (-43.9°)		

and $[\mu]_{D^{20}}^{calcld(ik)}$ is the Kirkwood term calculated as

$$[\mu]_{D^{20}}^{calcld(ik)} = \{288 \pi^2 N / \lambda^2\} \{(n^2 + 2)/3\} g_{ik} \quad (3)$$

where N is the Avogadro number, λ is the wave length used, and n is the refractive index of the medium (solution or pure liquid). If each one of the substituent groups (unit groups formed by division of the molecule) has its own optical axis of cylindrical symmetry

$$g_{ik} = \frac{1}{6} \alpha_i \alpha_k \beta_i \beta_k G_{ik} \bar{R}_{ik} (\bar{b}_i \times \bar{b}_k) \quad (4)$$

$$G_{ik} = \frac{1}{R_{ik}^3} \left[\bar{b}_i \cdot \bar{b}_k - 3 \frac{(\bar{b}_i \cdot \bar{R}_{ik})(\bar{b}_k \cdot \bar{R}_{ik})}{R_{ik}^2} \right] \quad (5)$$

$$\bar{R}_{ik} = \bar{R}_k - \bar{R}_i \quad (6)$$

where α_i (the mean polarizability of the group i) = $(3/4\pi N) \cdot \sum_s A_s$ (A_s is the value of the atomic refraction); β_i (the anisotropy ratio of polarizability of group i) = $\{\alpha_{11}^{(i)} - \alpha_{22}^{(i)}\} / \alpha_i$; and $\alpha_{11}^{(i)}$ and $\alpha_{22}^{(i)}$ are components of α_i parallel to \bar{b}_i and perpendicular to \bar{b}_i ; and β_i is computed from the depolarization degree Δ of light scattered by vapor; and where \bar{b}_i (the unit vector along the optical symmetry axis of group i) and \bar{R}_i (the vector distance from the center of mass of the molecule to the optical center of group i) are determined from the geometry of the molecule.

Kirkwood located the optical center of the OH group at its oxygen atom and the optical center of the CH₃ group at its carbon atom. However, the author believes that the optical center of the OH group is at the center of mass¹⁰ of the ¹C-(OH)¹¹ bond and that the optical center of the CH₃ group is at the center of mass of the ¹C-(CH₃) bond. In this article, these two methods of locating the optical center of a group are compared.¹³

The methylcyclohexanols and their observed molecular rotations are listed in Table I.

(10) Reference *13 in the previous paper.⁵

(11) ¹C atom means the C atom which constitutes the ring (ref. *15 in the previous paper⁵).

(12) Owing to its symmetry, the central group (the cyclohexane ring) does not appear in the calculations. Moreover, because of the small polarizabilities of the H atom and the C-H bond, the $[\mu]_{D^{20}}^{obs}$ value caused by the dynamic coupling effect of the H atom can be neglected.

(13) Given in parentheses are the values computed by assuming that the optical center of the OH group is at its oxygen atom and that the optical center of the CH₃ group is at its carbon atom.

(14) All of the $[M]_{D^{20}}$ values were obtained with the homogeneous liquid compounds. Since $\partial[M]_{D^{20}}/\partial T$ is small, the $[M]_{D^{20}}$ value of (+)-*cis*-3-methylcyclohexanol was assumed to be equal to its $[M]_{D^{18}}$ value.

(15) (OH)^{1β} refers to the OH group which has a β -orientation at the 1-position of the ring (that is, above the ring in Fig. 1; cf. L. F. Fieser, *J. Am. Chem. Soc.*, **72**, 623 (1950)).

(16) G. A. C. Gough, H. Hunter, and J. Kenyon, *J. Chem. Soc.*, 2052 (1926).

(17) W. Hückel and J. Kurz, *Ber.*, **91**, 1290 (1958).

(18) D. S. Noyce and D. B. Denny, *J. Am. Chem. Soc.*, **74**, 5912 (1952).

Several brackets appearing near the middle of the table indicate unit groups in a given methylcyclohexanol; any pair of these unit groups in the same bracket can interact to produce their own partial rotation.

Since spectral studies in the infrared indicate that there is no intramolecular hydrogen bonding between the OH and CH₃ groups in methylcyclohexanol,¹⁹ the most stable configuration should be the one with two equatorial unit groups.

To simplify computations, a cyclohexane derivative is assumed to have the following characteristics: the length of a C-C bond is 1.54 Å.; the length of a C-O bond is 1.42 Å.; the valency angle of a C atom is 109° 28'; the optical axis of cylindrical symmetry of an OH group is directed along the ¹C-OH bond and that of a CH₃ group along the ¹C-CH₃ bond; the mean polarizability, α , of the OH group is 1.04×10^{-24} cc.²⁰ and the α of the CH₃ group is 2.27×10^{-24} cc.²⁰; the anisotropy ratio, β , of both the OH group and the CH₃ group is 0.35²¹; the refractive indices, n , of the methylcyclohexanols are the same, namely 1.46.

Computations.—The $[\mu]_{D^{20}}^{calcld}\{3/(n^2 + 2)\}$ values (caused by the dynamic coupling effect between any two members of the unit groups) are calculated by eq. 3. These computed values are included in Table II.

TABLE II

	C1 Conformation					
	(CH ₃) ^{4β}	(CH ₃) ^{4α}	(CH ₃) ^{3β}	(CH ₃) ^{3α}	(CH ₃) ^{2β}	(CH ₃) ^{2α}
(OH) ^{1α}	0	0	G	0	0	E
(OH) ^{1β}	0	0	0	-F	E	-E
	1C Conformation					
	(CH ₃) ^{4α}	(CH ₃) ^{4β}	(CH ₃) ^{3α}	(CH ₃) ^{3β}	(CH ₃) ^{2α}	(CH ₃) ^{2β}
(OH) ^{1β} - ²²	0	0	-G	0	0	-E
(OH) ^{1α}	0	0	0	F	-E	E
	$[\mu]_{D^{20}}^{calcld}\{3/(n^2 + 2)\}^{14}$ (= (i) × (k))			$[\mu]_{D^{20}}^{obs}$ ¹⁴ (= (i) Λ (k))		
E	25.30 (13.35)			43.9 (43.9)		
F	4.20 (3.70)			7.3 (12.2)		
G	4.04 (3.34)			7.0 (11.0)		

Since there are only two unit groups ((OH)^{1β} and (CH₃)^{2α}) in (-)-*trans*-2-methylcyclohexanol, the $[\mu]_{D^{20}}^{obs}$ value in the molecule is limited to (OH)^{1β} Λ (CH₃)^{2α}²³ and this leads directly to $[M]_{D^{20}}$ of (-)-

(19) J. Meinwald and J. A. Yankeelov, Jr., *ibid.*, **80**, 5266 (1958).

(20) Landolt-Börnstein, "Physikalisch-Chemische Tabellen," Hw. 2, p. 985.

(21) Landolt-Börnstein, "Physikalisch-Chemische Tabellen," 5th Ed., Eq. 2, p. 90, 91.

(22) 1β means the 1β in the 1C conformation, and so on (ref. 15; Fig. 1).

(23) (OH)^{1β} Λ (CH₃)^{2α} means $[\mu]_{D^{20}}^{obs}$, caused by the dynamic coupling effect between (OH)^{1β} and (CH₃)^{2α}. (OH)^{1β} × (CH₃)^{2α} means the corresponding $[\mu]_{D^{20}}^{calcld}\{3/(n^2 + 2)\}$, and so on (ref. *10 in the previous paper⁵). By using eq. 2 here, (OH)^{1β} Λ (CH₃)^{2α} = (OH)^{1β} × (CH₃)^{2α} $\{OH^1CH_3/(n^2 + 2)/3\}$ (ref. *32 in the previous paper⁵).

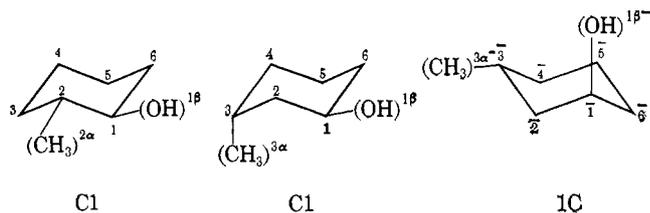


Figure 1.

trans-2-methylcyclohexanol, $-43.9 \equiv \Sigma[\mu]_{D^{20}}^{20}_{\text{obs}}$ of
 (-)-*trans*-2-methylcyclohexanol = $(\text{OH})^{1\beta} \wedge (\text{CH}_3)^{2\alpha}$
 $= (\text{OH})^{1\beta} \times (\text{CH}_3)^{2\alpha} \zeta_{\text{OH}\zeta_{\text{CH}_3}} \{ (n^2 + 2)/3 \}^{23} = -25.30$
 $\zeta_{\text{OH}\zeta_{\text{CH}_3}} \{ (n^2 + 2)/3 \}^{24}$

$$\therefore \zeta_{\text{OH}\zeta_{\text{CH}_3}} = 1.7352 \{ 3/(n^2 + 2) \} \quad (7)$$

or

$$\zeta_{\text{OH}\zeta_{\text{CH}_3}} \{ (n^2 + 2)/3 \} = 1.7352 \quad (7')$$

The values of E , F , and G for $[\mu]_{D^{20}}^{20}_{\text{obs}}$ in Table II can be obtained by multiplying those for $[\mu]_{D^{20}}^{20}_{\text{calcd}}$ $\{ 3/(n^2 + 2) \}$ by the value of $\zeta_{\text{OH}\zeta_{\text{CH}_3}} \{ (n^2 + 2)/3 \}$ given in eq. 7'.

(24) Table 11 is used.

Next, as seen in Table I, unit groups in (-)-*trans*-3-methylcyclohexanol of C1 conformation are $(\text{OH})^{1\beta}$ and $(\text{CH}_3)^{3\alpha}$ (Fig. 1). Therefore, $\Sigma[\mu]_{D^{20}}^{20}_{\text{obs}}$ of (-)-*trans*-3-methylcyclohexanol of C1 conformation = $(\text{OH})^{1\beta} \wedge (\text{CH}_3)^{3\alpha} = -F = -7.3$.²⁴ Similarly, $\Sigma[\mu]_{D^{20}}^{20}_{\text{obs}}$ for all of the methylcyclohexanols are calculated and given in the fourth column of Table I. As shown in the fifth column of Table I, even in the cases of (-)-*trans*- and (+)-*cis*-3-methylcyclohexanols where there are no adjacent substituents in the molecules, the values of $[M]_{D^{20}}$ are not zero, and yet these observed values are nearly equal to the calculated values, $\Sigma[\mu]_{D^{20}}^{20}_{\text{obs}}$. The observed value of $[M]_{D^{20}}$, -15.3° , of (-)-*cis*-2-methylcyclohexanol indicates the presence of 32.6% of C1 conformation (an equatorial OH and an axial CH_3) and 67.4% of 1C conformation (an axial OH and an equatorial CH_3) in the equilibrium state.²⁵ This is compatible with the anticipation that, owing to a larger volume of the CH_3 group, the repulsion between a CH_3 group and the cyclohexane ring is stronger than the repulsion between an OH group and the cyclohexane ring. The facts may indicate the application of the PM method to methylcyclohexanol.

(25) By his own method of calculation of $[M]_{D^{20}}$, Brewster reached the same conclusion (cf. J. H. Brewster, *J. Am. Chem. Soc.*, **81**, 5483 (1959)).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND LAWRENCE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIF.]

The Biosynthesis of Nicotine in *Nicotiana glutinosa* from Carbon-14 Dioxide¹

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Plants of *Nicotiana glutinosa* were grown in an atmosphere containing ¹⁴CO₂ for periods varying from 2 hr., the shortest time at which incorporation of radioactivity into nicotine could be detected, to 12 hr. The nicotine, isolated separately from the root and aerial portions, was degraded, and the activity in the pyridine ring, the N-methyl group, and carbon-2' of the pyrrolidine ring was determined. These data were correlated in terms of (1) the rate of incorporation of CO₂ into nicotine, (2) the site of nicotine syntheses, (3) the relative rate of N-methyl syntheses, and (4) the relative rate of syntheses of the pyridine and pyrrolidine rings. The conclusions thus reached have been compared with those in the literature derived from grafting experiments and from feeding precursors other than CO₂. Evidence is presented for independent nicotine synthesis in both root and aerial portions, and some questions are raised concerning the glutamate-symmetrical intermediate hypothesis for pyrrolidine ring biosynthesis.

Introduction

The biosynthesis of nicotine, and of related tobacco alkaloids, probably has been more extensively studied than that of any other alkaloid. The results and conclusions in this area have been summarized in three comprehensive reviews³⁻⁵ that have appeared recently. These results have been obtained almost exclusively by the now familiar technique of feeding various *Nicotiana* species with potential precursors which are isotopically labeled. The nicotine isolated is degraded to determine the site, if any, of incorporated label. Interesting results have been obtained in particular by feeding nicotinic acid,⁶ various amino acids related to glutamic acid,⁷⁻⁹ the usual N-methyl precursors,¹⁰ and glycerol

and organic acids related to the Krebs cycle.¹¹⁻¹⁴ These results subsequently will be discussed in detail as they relate to our data.

Another approach to the study of alkaloid biosynthesis is by exposure of the intact plant to radioactive carbon dioxide. In this case, the role of carbon dioxide as precursor is obvious; the question becomes one of rate of incorporation. For this approach to be of any value, *de novo* alkaloid synthesis from carbon dioxide must be sufficiently rapid to allow a differential labeling pattern among the various carbon atoms of the alkaloid. At least in *Papaver somniferum* this has been the case^{15,16}

(1) Sponsored in part by the United States Atomic Energy Commission.
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(8) E. Leete, *Chem. Ind. (London)*, 537 (1955); *J. Am. Chem. Soc.*, **80**, 2162 (1958).

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