[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY]

The Alkaline Hydrolysis of Scopolamine Methoxymethochloride: A New Route to Scopine

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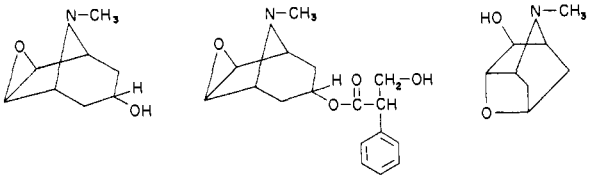
Scopolamine (II) has been converted to scopolamine methoxymethochloride which on hydrolysis and subsequent removal of the methoxymethyl group gives pure scopine in high yield.

Scopine (I), the true basic moiety of the alkaloid scopolamine (II), was obtained for the first time in 1923 by Willstätter and Berner.[2,3] Although inspection of the structural formulas of these two substances might lead to the conclusion that simple acid or base hydrolysis of II should lead to I, it has been the general experience that a rearrangement product, *dl*-scopoline (III), is obtained from all such hydrolysis attempts.[4] The technique successfully employed by Willstätter used only the buffer solution of a then customary ammonia–



I II III

ammonium chloride, pancreatic lipase, and olive oil hydrolysis procedure. This system resulted in a very slow hydrolysis and gave rise to a mixture of unchanged scopolamine, scopoline and scopine. Pure scopine was obtained from the mixture only after a tedious separation.

The interest of the authors in the possibilty of oxidizing scopine to the unknown ketone, scopinone, necessitated larger supplies of pure, crystalline scopine than could be conveniently prepared by the Willstätter technique. We have therefore sought a new route to scopine.

Recently, Moffett and Garrett observed that scopolamine methobromide hydrolyzes rapidly in barium hydroxide solution to give *scopine methobromide* rather than the rearranged scopoline derivative.[5] This striking result suggests either that the conversion of quaternary salts of scopine to the corresponding scopoline derivatives must occur less readily than does the conversion of free scopine into scopoline, or that the acceleration of the ester hydrolysis allows the initially formed scopine derivative to survive unrearranged until it can be isolated. In either case, it might be expected that a suitable quaternary salt of scopolamine could be prepared, such that free scopine could be regenerated from the quaternary scopine salt liberated by a rapid ester hydrolysis. By using chloromethyl methyl ether as the quaternizing agent, this expectation has been realized, and a simple, direct conversion of scopolamine into scopine has been achieved. A 90–100% yield of I, based on the scopolamine hydrolyzed, is obtained by this process.

Scopolamine methoxymethochloride (IV) was prepared by adding an ethereal solution of scopolamine to chloromethyl methyl ether in ethyl ether. The salt IV was extremely hygroscopic. Brief exposure to air converted the powdery solid to a viscous oil. Consequently, IV was not characterized. Barium hydroxide hydrolysis of IV, followed by acid cleavage of the methoxymethyl group and continuous extraction with ether gave scopine in almost quantitative yield. The product was contaminated with neither unchanged scopolamine nor scopoline (evidenced by the complete absence of ester carbonyl absorption and characteristic scopoline maxima at 7.16, 8.56, 8.95, 11.08, 11.20, 12.95 and 14.30 μ in the infrared). The product obtained by this method, when crystallized from pentane–hexane, gave well-formed colorless needles, m.p. 75–76° (reported for scopine, m.p. 76°[2]). The product was converted into a methobromide which had a melting point and infrared spectrum identical with those of an authentic sample of scopine methobromide prepared by the Moffett and Garrett procedure.[5]

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Experimental

Scopine (I).—Scopolamine (11.2 g., 0.037 mole) in 50 ml. of ether was dried over sodium carbonate.

Potassium hydroxide (2.0 g.) was ground to a powder under ether and transferred to a 500-ml. 3-neck flask. Ether was added to make the total volume approximately 200 ml. Chloromethyl methyl ether (5.95 g., 0.074 mole) was weighed into ether and added to the ether over potassium hydroxide. The solution of scopolamine was then added dropwise with vigorous stirring. The system was kept under dry nitrogen. After stirring 2 hours, barium hydroxide (6.5 g., 0.037 mole) in 170 ml. of water was added. Stirring was continued for 10 minutes, then the two layers were separated. The aqueous solution was extracted several times with small portions of ether. This extract, which contains the unreacted scopolamine, was dried over anhydrous magnesium sulfate. The aqueous solution was left standing at room temperature for 2 hours to effect complete ester hydrolysis. At the end of this time, the solution was acidified to *p*H 1–2 with 6*N* sulfuric acid to remove the methoxymethyl group and left for 2 hours at room temperature. After extraction with several small portions of ether to remove neutral material, the solution was made basic (*p*H 10–11) with saturated aqueous sodium carbonate and continu-

(1) American Viscose Corporation Fellow, Summer, 1956.

(2) R. Willstätter and E. Berner, *Ber.*, **56**, 1079 (1923).

(3) It is interesting to note that both scopine and scopolamine have recently been obtained by total synthesis by G. Fodor, J. Tóth, I. Koczer, P. Dobó and I. Vincze, *Chemistry & Industry*, 764 (1956).

(4) R. H. F. Manske and H. C. Holmes, "The Alkaloids," I, Academic Press, Inc., New York, N. Y., 1950, pp. 302–307.

(5) R. B. Moffett and E. R. Garrett, THIS JOURNAL, **77**, 1245 (1955).

ously extracted with ether. Evaporation of the ether from the first ether extraction gave scopolamine (4.2 g.) as a cloudy viscous oil.

Evaporation of the ether from the continuous extraction, after drying over anhydrous magnesium sulfate, gave 3.35 g. of scopine as a clear viscous oil. This is a 96% yield based on unrecovered scopolamine.

The colorless oil was taken up in excess pentane and treated with 0.1 g. of charcoal. After removal of the charcoal, hexane was added and the solution was concentrated by gentle warming. The solution was cooled to room temperature and then in the refrigerator overnight. This gave crystalline scopine, m.p. 73–75°. One further crystallization from pentane gave colorless needles, m.p. 76°.

Solid scopine as a Nujol mull showed the following infrared maxima: 3.04, 7.52, 7.65, 7.80, 8.07, 8.23, 8.33, 8.68, 8.85, 9.28, 9.60, 9.77, 10.05, 10.18, 10.60, 11.50, 11.80, 12.29, 13.70 and 14.11 μ.

Scopine in aqueous solution formed a picrate in good yield when treated with a methanolic solution of picric acid. The picrate had m.p. 242–243° (identical with the melting point of authentic scopoline picrate). A mixture melting point with authentic scopoline picrate showed no depression. The infrared spectra of scopine picrate and scopoline picrate were, however, distinctly different, each spectrum containing maxima totally absent in the other. The conversion of scopine picrate to scopoline picrate on heating to the melting point is not surprising in view of the report by Moffett and Garrett that scopine methobromide rearranges to scopoline methobromide on heating to the melting point.[5]

Scopine Methobromide.—A solution of scopine in methanol was treated with excess methyl bromide and left overnight at 0°. Scopine methobromide was then collected in nearly quantitative yield (m.p. 294–296° dec.).

Scopoline (III).—Scopolamine, dissolved in excess 10% potassium hydroxide, gave scopoline as a clear viscous oil in 98% yield after 17 hours reflux.

Scopoline showed the following infrared maxima: 2.97, 3.47, 6.86, 6.98, 7.16, 7.41, 7.54, 7.65, 7.74, 8.04, 8.21, 8.35, 8.56, 8.95, 9.31, 9.49, 9.60, 9.68, 9.82, 9.98, 10.20, 11.08, 11.20, 11.44, 12.00, 12.21, 12.50, 12.95 and 14.30 μ.

Scopoline picrate, obtained by mixing an aqueous solution of picric acid an an aqueous solution of scopoline, had m.p. 242–243° dec.

ITHACA, NEW YORK

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Variation of the Michaelis Constant in Polyphenol Oxidase Catalyzed Oxidations: Substrate Structure and Concentration

BY LLOYD L. INGRAHAM

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A study is reported of the variation of the oxygen Michaelis constant with the structure of the hydrogen donor in a system catalyzed by polyphenol oxidase. Three hydrogen donors, catechol, chlorogenic acid and caffeic acid, were chosen for this study. From the assumption that the rate constants governing the equilibrium between enzyme and oxygen are independent of hydrogen donor structure and the fact that the Michaelis constant for oxygen is highly dependent upon the maximum velocity for three different hydrogen donors, the conclusion is drawn that the oxygen Michaelis constant is not an equilibrium constant. A study is also reported of the variation of the oxygen Michaelis constant with the hydrogen donor concentration. This effect had been predicted previously from theoretical considerations. The Michaelis constants approach a value of ~1.5% oxygen at low values of the hydrogen donor concentration regardless of the hydrogen donor structure. These facts are consistent with the ideas that the equilibrium constant between enzyme and oxygen is ~1.5% oxygen and also that the enzyme combines with oxygen before it does with the hydrogen donor.

In a recent paper[1] the kinetics for a two-substrate enzyme-catalyzed system were discussed. This discussion showed theoretically how the Michaelis constant for one substrate may vary with the concentration of the other substrate. Previous variations of Michaelis constants have been reported for glucose oxidase,[2] lipoxidase[3] and dextransucrase.[4] The purpose of this paper is to report a study of the variation of the Michaelis constant for oxygen with both structure and concentration of the other substrates, the hydrogen donors, in the polyphenol oxidase catalyzed system and also a discussion of the significance of these variations in understanding the mechanism of the polyphenol oxidase catalyzed aerobic oxidation of catechol.

Measurements of Michaelis Constants.—The Michaelis constants for the substrate oxygen were determined from the curvature in the plots of the consumption of oxygen *vs.* time as measured by means of a rotating polarized electrode.[5] The decrease in rate of oxygen consumption with time may be the result of either or both the diminution of the oxygen supply in solution or a decrease in catalytic activity of the enzyme from reaction-inactivation.[6,7] The hydrogen donor is kept at a constant concentration by the excess of ascorbic acid in solution. One may calculate when reaction-inactivation is important in affecting the curvature as follows:

The rate of consumption of oxygen has a Michaelis dependence on oxygen concentration[8]

$$\frac{d(O_2)}{dt} = -\frac{k_0(E)(O_2)}{(O)_2 + K_m} \qquad I$$

where k_0 is the rate constant for oxidation, K_m is the Michaelis constant and (E) is the enzyme concentration. In addition, the rate of disappearance of enzyme

$$\frac{d(E)}{dt} = -\frac{k_1(E)(O_2)}{(O)_2 + K} \qquad II$$

has a similar dependence on oxygen concentration.[8] A combination of equations I and II gives

$$k_1\, d(O_2) = k_0 d(E) \qquad III$$

(1) L. L. Ingraham and B. Makower, *J. Phys. Chem.*, **58**, 266 (1954); see also J. Z. Hearon, *Physiol. Revs.*, **32**, 499 (1952).

(2) H. Laser, *Proc. Roy. Soc.* (*London*), **140B**, 230 (1952).

(3) A. L. Tappel, P. D. Boyer and W. O. Lundberg, *J. Biol. Chem.*, **199**, 267 (1952).

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(8) L. L. Ingraham, *ibid.*, **77** 2875 (1955).