2. The pigment, which is responsible for all or most of the red color of strawberries, is believed to be pelargonidin 3-monoglucoside. The possible presence of two isomers of pelargonidin in the isolated material is discussed.

3. Absorption curves for the glucoside and the aglucone are presented.

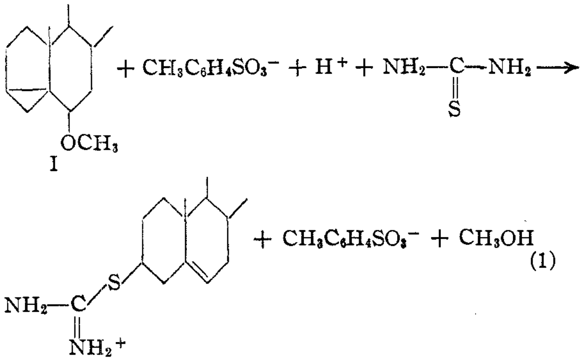
GENEVA, N. Y. RECEIVED MAY 26, 1948

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Mechanism of Formation of Cholesterylisothiuronium Salts from *i*-Cholesteryl Methyl Ether[1a]

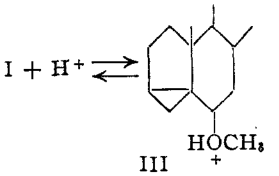
BY R. G. PEARSON, LEE A. SUBLUSKEY AND L. CARROLL KING

In recent papers from this Laboratory[1b,1c] it was shown that *i*-cholesteryl methyl ether (I) reacts with thiourea and *p*-toluenesulfonic acid in alcoholic solution to give cholesterylisothiuronium tosylate (II).



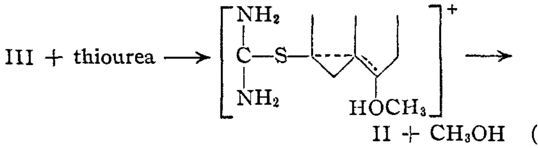
II

Since there is a change in the nature of the ions present during the course of the reaction it is possible to make a kinetic study by measuring the corresponding change in the electrical conductivity. The results of such a kinetic study indicate that the reaction is first order with respect to each reactant. Two possible mechanisms are consistent with the experimental data: (a) reaction between I and hydrogen ion resulting in an oxonium complex (III),[1,2] which then reacts with nucleophilic thiourea in an abnormal type of displacement (3); or (b) formation of III followed by reversible ionization into methanol and a carbonium ion (IV) which can then react with thiourea at the 3 position as shown in (5).

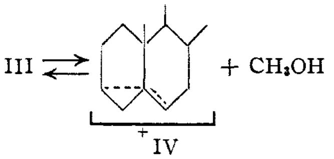


(2)

III



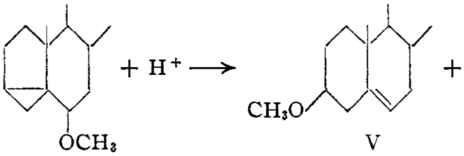
(3)



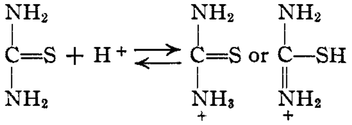
(4)

$$\text{IV} + \text{thiourea} \longrightarrow \text{II} \qquad (5)$$

The kinetics are complicated by the simultaneous acid catalyzed rearrangement of the isomeric ether (I) to cholesteryl methyl ether (V), which will not react further to give the product (II), and by the formation of a thiuronium ion (VI) from thiourea and hydrogen ion.



(6)



$$\underset{\text{NH}_2}{\overset{\text{NH}_2}{\text{C}}}{=}\text{S} + \text{H}^+ \rightleftharpoons \underset{\overset{+}{\text{NH}_3}}{\overset{\text{NH}_2}{\text{C}}}{=}\text{S} \text{ or } \underset{\overset{+}{\text{NH}_2}}{\overset{\text{NH}_2}{\text{C}}}\text{—SH} \qquad (7)$$

VI

The rate of reaction (6) was determined directly by measurement of the change in optical rotation in the absence of thiourea. In the presence of sufficient excess of thiourea this side reaction is of minor importance.

The concentration equilibrium constant for reaction (7) in dry methanol was determined by independent means and will be reported elsewhere.[3] Its numerical value of *ca.* 15 offers evidence that in the presence of excess thiourea much of the hydrogen ion is tied up as thiuronium ion (VI).

Experimental

Materials.—*i*-Cholesteryl methyl ether was prepared from cholesteryl *p*-toluenesulfonate by the method of Stoll,[4] m. p. 78–79°, $[\alpha]^{23}\text{D}$ 51.2°. *p*-Toluenesulfonic acid monohydrate was purified by several recrystallizations from concentrated hydrochloric acid, m. p. 104–105°. Thiourea was purified from a C.P. grade by recrystallizing

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[1a] Presented before the Organic Division of the American Chemical Society, Chicago meeting, April, 1948; (b) King, Dodson and Subluskey, THIS JOURNAL, **70**, 1176 (1948); (c) King, *ibid.*, **70**, 2685 (1948).

[2] Meyer, Ph.D. Thesis, Northwestern University, 1943.

[3] Pearson and Tucker, forthcoming publication.

[4] W. Stoll, *Z. physiol. Chem.*, **207**, 147 (1932).

from ethanol, m. p. 177°. Methanol was dried according to the method of Lund and Bjerrum.[5]

Methods.—Weighed samples of thiourea in 10- to 40-fold excess were dissolved in 25 cc. of an 0.01 *M* methanolic *i*-ether solution and then brought to the reaction temperature of 32.5°. The reaction was initiated by adding an amount of *p*-toluenesulfonic acid equivalent to the *i*-ether from a 0.05 *M* methanolic solution of the acid. A portion was then withdrawn into a Washburn type conductance cell, tightly stoppered and placed in a thermostat set at 32.50 ± 0.02°. Readings of the resistance were made over a period of several hours using a Jones–Josephs conductance bridge. A final reading of the resistance at equilibrium was made after two or three days.

The direct measurement of reaction (6) was carried out with 0.01 *M* methanolic *i*-ether solutions of varying acid concentrations by employing a 2-dcm. polarimeter tube and thermostating to 32.5° with the exception of short intervals required for rotation measurements. Only one or two readings were made on each solution. For initial measurements readings were made in the absence of acid.

Calculations and Results

If a is the initial concentration of *i*-ether (I) or acid and x is the concentration of product formed then it can be shown[6] in terms of resistance that

$$\frac{a}{a-x} = \frac{(R_e - R_0)R}{R_0(R_e - R)} \quad (8)$$

where R is the resistance for any value of x, R_0 is the initial resistance, and R_e is the resistance at equilibrium. Since in the reaction the mobile hydrogen ion or thiuronium ion is replaced by a much less mobile ion (II), R_e is greater than R_0 by a factor of some 50%. Straight lines resulted, as in Fig. 1, when $R/(R_e - R)$ was plotted against time. This is in agreement with a pseudo second order reaction whose apparent rate constant, k', can be found by dividing the slope of the line by the intercept and by a, the initial concentration, since the usual second order rate equation

$$t = (1/ak')[a/(a-x)] - 1/ak'$$

becomes

$$t = (1/ak')[(R_e - R_0)/R_0][R/(R_e - R)] - 1/ak'$$

in terms of resistances. The values thus found are recorded in Table I, and are fairly constant for a several-fold increase of thiourea. Because of the existence of equilibrium (7) the reaction, nevertheless, is not independent of the thiourea concentration since a doubling of thiourea increases the rate by a factor of two but likewise decreases it almost an equal amount by decreasing the hydrogen ion concentration.

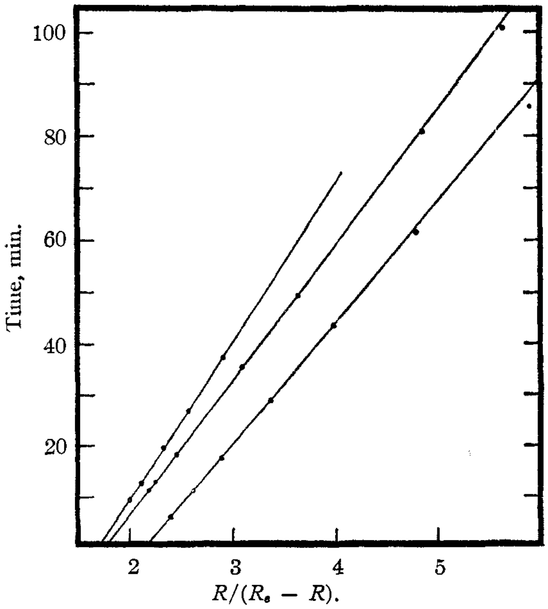

Fig. 1.—Left to right: 0.174, 0.304 and 0.087 *M* thiourea + 0.0083 *M* *i*-ether and H$^+$.

TABLE I

p-TOLUENESULFONIC ACID AND *i*-ETHER, 0.0083 MOLAR

Thiourea, molar	k'	$k'([T] + K)/K[T]^a$	k_1 liters²/moles²-min.
0.087	2.26	61.5	43.5
.174	2.39	50.3	41.2
.304	2.62	48.3	43.0

[a] The thiourea concentration is corrected for thiuronium salt formation.

In a detailed analysis, if [T] is the thiourea concentration and [TH$^+$] is the thiuronium ion concentration then

$$K = \frac{[T][H^+]}{[TH^+]} \quad (9)$$

where K is the concentration equilibrium constant for the reverse of reaction (7). It then follows that

$$[TH^+] + [H^+] = (a - x) = [H^+][(K + [T])/K] \quad (10)$$

Now if the formation of the cholesteryl isothiuronium ion from *i*-ether is first order in all three reactants then

$$\begin{aligned} dx/dt &= k_1[I][H^+][T] \\ &= k_1(a-x)^2\frac{K[T]}{[T] + K} = k'(a-x)^2 \quad (11) \end{aligned}$$

and the apparent rate constant k' will be equal to the expression $k_1K[T]/([T] + K)$ which will increase somewhat as [T] increases but for large values of [T] compared to K will have a constant value of k_1K. Since K is known,[3] (1/15), the values of k_1 can be found from the experimental constant k' and equation (10). These are recorded in Table I under the heading of $k'([T] + K)/[T]K$. With decreasing thiourea concentration these constants show a decided drift, an effect resulting from the side reaction (6). This can be shown by including (6) in the rate expression, assuming that it is first order in hydrogen ion and *i*-ether. Then

$$\begin{aligned} -d[I]/dt &= k_1[H^+][I][T] + k_2[H^+][I] \\ &= (a-x)^2\left[\frac{k_1K[T] + k_2K}{[T] + K}\right] \quad (12) \end{aligned}$$

If the formation of normal ether is a minor reaction, then $dx/dt = -d[I]/dt$, and consequently the rate constant k' can be identified with the total quantity $(k_1K[T] + k_2K)/([T] + K)$.

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[5] Lund and Bjerrum, *Ber.*, **64B**, 210 (1931).

[6] Pearson, THIS JOURNAL, **69**, 3100 (1947).

Substitution of known values of k_2 and K allows k_1 to be evaluated. Such results are substantially constant as shown in Table I and indicate that the side reaction is of the proper order of magnitude to explain the drift in the rate constants.

The rate constant k_2 was found by employing a pseudo first order rate expression

$$k_2 = \frac{1}{at} 2.303 \log \frac{(\alpha_0 - \alpha_e)}{(\alpha - \alpha_e)} \qquad (13)$$

the α's referring to measured rotations at the beginning, the end and at time t. Table II shows the results of these calculations.

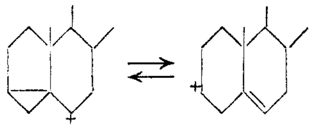
TABLE II

CONVERSION OF *i*-ETHER TO NORMAL ETHER

Acid, molar	Time, min.	$\log \frac{\alpha_0 - \alpha_e}{\alpha - \alpha_e}$	k_2 liters/mole-min.
0.0083	95	0.500	1.46
.0083	185	.996	1.49
.0102	53	.274	1.18
.0102	16	.094	1.32

Discussion

It is not possible to ascertain whether a carbonium ion is formed as an intermediate or whether the reaction proceeds by a nucleophilic displacement since both mechanisms could have the same dependence on thiourea concentration. If it is a nucleophilic displacement, then it is an unusual kind of S_N2 reaction in which the entering group attacks the 3 position forcing the electrons of the 3,5-cyclopropane bond to the 5,6 positions where in turn they displace a molecule of methanol. It resembles the abnormal type of allylic displacement which has been looked for by several investigators but never clearly demonstrated.[7] A carbonium ion intermediate is possible if the following statements are true: reaction of the ion at the 6 position with a nucleophilic reagent is much more rapid than at the 3 position; any *i*-cholesterylisothiuronium ion formed by reaction of thiourea at the 6 position is unstable and regenerates the carbonium ion[1]; reaction at the 3 position is sensitive to the reactivity of the entering group (that is, thiourea reacts more rapidly than methanol). It is not too improbable that all of these conditions hold true. Application of the steady state theory[8] then will give a rate dependent on the thiourea concentration as observed. The observed stereospecificity of reactions in the *i*-steroid series does not rule out a carbonium ion intermediate since resonance of the unsaturation electrons will tend to hold the original configuration.[9]


(14)

The function of hydrogen ion in the reaction is clearly to reduce the basicity of the group which is to be displaced from that of a methoxide ion to that of a molecule of methanol. The resemblance to the acid cleavage of benzyl and allyl ethers is more than a formal one since the combination of weakened carbon–oxygen bond and unsaturation (cyclopropane ring) in the β,γ position is necessary. The normal ether, for example, with unsaturation in the γ,δ position does not react in a similar way.

Whether these reactions show general acid or specific hydrogen ion catalysis could not be determined from these studies. It seems probable that an acid–base equilibrium such as shown in (1) is rapidly established and that a slower displacement or ionization follows. If this is true specific hydrogen ion catalysis would be expected.[10]

Summary

1. The reaction of *i*-cholesteryl methyl ether, hydrogen ion and thiourea to form a cholesterylisothiuronium salt is first order in each reactant.

2. The third order rate constant for this reaction at 32.5° has been measured as well as the second order rate constant for the acid catalyzed conversion of *i*-ether to normal ether.

3. The mechanism is either an abnormal S_N2 displacement involving the shift of a pair of electrons from the 3,5 to the 5,6 position, or goes by way of a carbonium ion which reacts slowly and discriminately at the 3 position and rapidly and reversibly at the 6 position.

EVANSTON, ILLINOIS RECEIVED MAY 6, 1948

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[7] (a) Roberts, Young and Winstein, THIS JOURNAL, **64**, 2157 (1942); (b) Hughes, *Trans. Faraday Soc.*, **34**, 185 (1938).

[8] See, for example, Branch and Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1941, ch. VII.

[9] Winstein and Adams, THIS JOURNAL, **70**, 838 (1948); Dodson, Ph.D. Thesis, Northwestern University, 1947.

[10] R. P. Bell, "Acid–Base Catalysis," Oxford University Press, London, 1941, Chapter VII.