recrystallized from ethanol) prepared from sodium diphenylmethide and ethylene chloride.[8]

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Fate of Selenium in the Isomerization of Oleic Acid

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Previous work in this laboratory on the selenium-catalyzed isomerization of *cis* -stilbene[2] and of *cis*-9-octadecenoic (oleic) acid[3] at 200° to their respective trans isomers established that the rate of conversion was essentially independent of the concentration of selenium at any particular initial concentration of selenium, despite the fact that the selenium slowly disappeared from the solid phase.

In the present paper, the fate of the selenium during the isomerization of oleic acid is examined and there are presented experimental data, consistent with the requirement that the concentration of the catalytically active species of selenium be essentially constant during the course of the isomerization.

EXPERIMENTAL

Three forms of the selenium catalyst are present during the isomerization of the oleic to elaidic acid at 200°: the original black, undissolved or bulk selenium; the active form of the selenium in solution, which on cooling, precipitates as the red modification; and the inactive species which remains in "solution" after cooling. In order to determine the approximate quantities of these three forms of selenium, pure oleic acid was heated to 200° for various time intervals using a fixed amount (0.2 weight percent) of black selenium powder.

Apparatus. The apparatus employed for the determinations consisted of a test tube reactor, equipped with an agitator passing through a glass-tube bearing in a rubber stopper, and having a side tube connected to a helium supply for maintaining an inert atmosphere. The reactor was heated by means of a salt bath controlled at 200° with a heating mantle connected through a variac to a Thermocap relay.

Procedure. The determinations were all carried out in the same manner. A typical experiment will be described:

Pure oleic acid, 40.017 g. was placed in the reactor and heated with stirring under a helium atmosphere. After the temperature had become adjusted to 200° ± 2°, the stopper was momentarily removed and 0.0800 g. of black selenium powder on a fragile glass boat was added. After replacing the stopper and starting agitation, the time was noted. After 12 minutes, the heating bath and agitator were

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(2) J. D. Fitzpatrick and M. Orchin, *J. Org. Chem.*, **22**, 1177 (1957).

(3) J. D. Fitzpatrick and M. Orchin, *J. Am. Chem. Soc.*, **79**, 4765 (1957).

quickly removed and a filter stick inserted. The contents of the reactor were quickly filtered while hot into a tared filter flask. The black selenium remaining in the reaction vessel was transferred to a weighed, fritted-glass, microfilter funnel with the aid of petroleum ether. Since the material on the filter contained bits of glass from the weighing boat, it was necessary to dissolve the selenium in the weighed mixture with concentrated nitric acid and wash and dry and reweigh the filter funnel to obtain the true weight. Using this procedure it was determined that 0.0390 g. of black selenium powder was present. On cooling the oleic-elaidic acid mixture in the filter flask, red selenium precipitated. The mixture was diluted with petroleum ether and filtered through a weighed, fritted-glass micro-filter funnel. After washing with petroleum ether and drying, 0.0192 g. of red selenium was secured. The weight of "inactivated" selenium which remained in "solution" was 0.0218 g., by difference. The results are semi-quantitative but sufficiently accurate to allow appropriate conclusions.

RESULTS AND DISCUSSION

The results of measurements with 0.2 weight percent initial selenium at 200° are shown in Table I.

Disappearance of the solid catalyst is rapid until a certain concentration level of the active form is obtained; solution by complexing occurs thereafter only as it is needed to maintain this concentration level against an inactivation reaction occurring concomitantly.

The selenium which precipitates in the red form on cooling is believed to represent the active species of the catalyst. Table I shows that the concentration of this form at 200° increases rapidly and then remains essentially constant (within the limits of accuracy of the analytical method used) during the reaction or until all the undissolved black form has disappeared. These data are in agreement with the pseudo first-order rate equation found for the reversible selenium-catalyzed interconversion of oleic (cis) and elaidic (trans) acids:

$$-\frac{d[\text{Oleic}]}{dt} = k_1'[\text{Oleic}] - k_2'[\text{Elaidic}]$$

TABLE I

RATE OF SOLUTION OF SELENIUM IN PURE OLEIC ACID, 200°, 0.2% INITIAL SELENIUM

Heating Time, (Min.)	Percent of Total Selenium		
	Undissolved black	Precipitated red	Inactive (by diff.)
3	89.0	8.5	2.5
7	58.7	27.7	13.6
12	48.7	24.0	27.3
15	34.2	30.5	35.3
25	19.0	34.7	46.3
35	0.0	6.0	94.0

where the k' values include a particular initial concentration of selenium. The rapid attainment of the (theoretically) constant value for the concentration of active selenium corresponds to the rapid equilibrium reaction between oleic acid and its pi complex with selenium, according to the mechanism previously advanced.[3]

The concentration of the inactive form of selenium increases at a constant rate giving essentially a straight line. This suggests that the inactivation reaction depends on the active species which is in constant concentration. Such an effect follows from the mechanism advanced previously, in which the inactivation of the catalyst was ascribed to an irreversible rearrangement of the pi complex to a new species presumably involving the formation of a carbon-selenium sigma bond.

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Isolation of an Alkaloid, Annuloline, from the Roots of *Lolium Multiflorum*[1,2]

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The roots of the annual rye grass (*Lolium multiflorum*) have been known for some time to contain a brilliant blue fluorescing pigment.[5] Seed analysts are able to distinguish annual rye grass seed from the morphologically similar perennial variety (*L. perenne*) by viewing the roots of the 8-day old seedlings under long wave ultraviolet light; the perenne roots do not fluoresce.[6]

The extraction of the fresh roots of the seedlings obtained from about 100 lbs. of seed of *L. multiflorum* has enabled us to isolate and crystallize the fluorescent principle which appears to be a weakly basic alkaloid. The tentative empirical formula, $C_{20}H_{19}NO_4$, best satisfies the presently available data. It seems likely that there are three methoxyl groups present per formula weight.

A variety of organic solvents such as ethanol, methylene chloride, carbon tetrachloride, ether, benzene, and petroleum ether serve to extract this substance. Since it is both an extremely weak base and only sparingly soluble in water it cannot be readily extracted with hydrochloric acid. Having no acidic (or phenolic) groups it is not extractable with alkali. The pigment is strongly fluorescent. In petroleum ether it fluoresces with an intensity about 13 times that of quinine sulfate in 0.1 N sulfuric acid on a weight basis when compared in a model 12B Coleman fluorophotometer fitted with B-1 and PC-1 filters. It can be readily detected on filter paper by virtue of its fluorescence. In general it exhibits high mobilities on being chromato-

graphed on paper with solvents of low water content. Acidification of solvents with hydrochloric acid or basification with ammonium hydroxide such as employed by Swain[7] as diagnostic procedures for discriminating between acidic (or enolic) and basic plant pigments did not influence the mobility of the pigment.

A hydrochloride can be obtained by the action of anhydrous hydrogen chloride on a solution of the alkaloid in ethanol or petroleum ether. The resulting salt is strongly acidic. Because the characteristic blue fluorescence of solutions of the alkaloid is not shown by the salt form it is possible to estimate from a plot of fluorescence vs. pH that the base is approximately 50% in the free form at pH 2.0.

By means of ultraviolet light as little as 0.01 mcg. of the alkaloid may be detected when applied to a 2-mm. diameter area on Whatman No. 1 filter paper. Exposure of the spot to fumes of hydrochloric acid abolishes the blue fluorescence and the spot now glows yellow-green. In sufficient amounts the hydrochloride is visible as a green-yellow spot in visible light. Fumes of ammonia reverse this change. This simple test serves to distinguish the alkaloid from a number of naturally occurring fluorescent substances such as methyl anthranilate, anthranilic acid, terthienyl, various coumarins, flavanoids, and alkaloids.

A characteristic property of this alkaloid is the marked decrease in fluorescence which occurs in its solution in petroleum ether on the addition of various oxygen-containing solvents such as methanol, ethanol, *n*-propanol, ether, and acetone. Ethanol is remarkably effective in this respect. However its presence does not significantly alter the ultraviolet absorption spectrum as obtained with a petroleum ether solution of the alkaloid. The possibility that the quenching phenomenon is due to the high dielectric constants of the materials used is unlikely since a concentration of $0.12M$ of methanol is sufficient to cause a 50% loss of fluorescence in petroleum ether.

Workers in New Zealand[8-12] have isolated and characterized an alkaloid from the aerial portion of *Lolium perenne* (perennial rye grass) which they have named, perloline. Following this precedent we offer the name "annuloline" for the alkaloid isolated from the roots of the annual rye grass. Perloline and annuloline are clearly different compounds on the basis of their chemical and physical properties, analyses, fluorescent colors, absorption spectra,

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(5) G. Gentner, *Prakt. Blätter Pflanzenbau u. Pflanzenschutz*, **6**, 166 (1929).

(6) U. S. Dept. of Agriculture, *Testing Agricultural and Vegetable Seeds*, Government Printing Office, Washington, D. C. (1952), pp. 103-104.

(7) T. Swain, *Biochem. J.*, **53**, 200 (1953).

(8) R. E. R. Grimmet and J. Melville, *New Zealand J. Sci. Technol.*, **24B**, 149 (1943).

(9) R. E. R. Grimmet and D. F. Waters, *New Zealand J. Sci. Technol.*, **24B**, 151 (1943).

(10) I. Reifer and N. O. Bathurst, *New Zealand J. Sci. Technol.*, **24B**, 155 (1943).

(11) F. B. Shorland, *New Zealand J. Sci. Technol.*, **24B**, 159 (1943).

(12) E. M. Clare and I. M. Morice, *New Zealand J. Sci. Technol.*, **27B**, 36 (1945).