[CONTRIBUTION FROM THE HORMEL INSTITUTE, UNIVERSITY OF MINNESOTA]

Stabilization of Autoxidizable Materials by Means of Inclusion[1]

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Adducts of α-dextrin (cyclohexaamylose), β-dextrin (cycloheptaamylose) and deoxycholic acid were prepared, with linoleic acid, linolenic acid, methyl linolenate, cinnamaldehyde and vitamin A palmitate. They were found to be very resistant to autoxidation. The conventional procedure of preparing choleic acids yielded stable products with linolenic acid and vitamin A palmitate. The products obtained from dextrins with linoleic acid, linolenic acid and cinnamaldehyde needed purification. A heat treatment under high vacuum was found to be reliable for obtaining stable adducts free of autoxidizable contamination. The principle of inclusion stabilization appears to be established by these examples and by the previous work on fatty acid stabilization by means of urea.

Several years ago it was discovered that autoxidizable fatty acids are stabilized when included in urea.[2] Although a solid may autoxidize more slowly than a liquid, other factors being equal, this did not explain the complete stabilization observed. Two possible explanations were advanced: (1) the crystal lattice of the adduct offers a barrier against the free penetration of oxygen, and (2) the specific crystal structure of urea compounds should mechanically prevent any chain mechanism.[3] Both suggestions should apply to all inclusion compounds and would be strengthened if this stabilizing effect were characteristic of other adducts besides those of urea. Practical aspects also made the extension of these studies desirable. With urea, stabilization by inclusion is restricted to fatty acids or, more generally, to essentially unbranched molecules since only straight chains preferentially react with urea. Secondly, potential physiological uses of inclusion compounds make desirable the choice of "host molecules" other than urea. Although in recent nutritional experiments, the urea compounds of fatty acids have been used successfully,[4] urea is not a constituent of the human diet and some physiological effects of urea have been reported.[5]

It was expected that the stabilizing effect would be most pronounced and most easily demonstrated with inclusion compounds of the channel type.[6] In the studies described here, the compounds of various autoxidizable substances with α- and β-dextrin and deoxycholic acid, all belonging to the channel type, are tested in regard to their autoxidation. The results suggest that any autoxidizable substance, as long as it is included, will be greatly stabilized. The mode of autoxidation inhibition is different from that of chain inhibitors such as hydroquinone or similar antioxidants. This is proven by the fact that impure adduct preparations will autoxidize rapidly, although the host molecules, *i.e.*, dextrin or deoxycholic acid, are present in large amounts. Of the explanations cited above, the prevention of a chain mechanism when the molecules are fixed by the host structure seems to be more pertinent. Concerning the prevention of diffusion of gases through the host structure, we cannot be so definite. Obviously all compounds prepared here can bind and release considerable amounts of gas. Whether this is a surface phenomenon or true inclusion is not yet clear. The inclusion compounds described here are not convenient for studying this phenomenon.

Exposed surface is one of the factors determining the rate of autoxidation. The large surface of the powdery adducts compares unfavorably with that of the original oils in this respect. Nevertheless, the rate of autoxidation of the oils in amounts approximating those in the adducts were taken as control rates.

Experimental

Materials.—The Schardinger α- and β-dextrins were prepared in this Laboratory combining the experiences published by others.[7,8] Deoxycholic acid, a commercial product, was treated with charcoal in methanol. It was further purified as described by Wieland and Sorge.[9] Fatty acids or esters were obtained from the Hormel Foundation. Commercial cinnamaldehyde was redistilled to obtain a fraction, b.p. 125–126° (18 mm.). Vitamin A palmitate was "Roche synthetic 97%."

Procedures.—For easy sampling and storing, solid autoxidizable materials were kept in a ground joint tube, both ends of which were fitted with an adapter bearing a stopcock. This permitted alternately flushing with purified nitrogen and evacuating. Similar tubes served for storage of liquids. The reactions were carried out under nitrogen either in sealed test-tubes or in erlenmeyer-type flasks, adapted for filling, heating, shaking and filtering in an inert atmosphere. Weighing and transfers were done without special protection. The amounts of the autoxidizable fatty acids used were measured by pipet.

The autoxidation tests were performed in a Warburg apparatus, the manometers of which were filled with silicone fluid. The materials were put in the vessels under nitrogen and then placed in the bath at 37 ± 0.2°. The initial reading was taken after four minutes of flushing with oxygen. These rather extreme conditions shortened the time necessary for evaluating the stability of the substances.

β-Dextrin–Linoleic Acid.—β-Dextrin (8 g.) was dissolved in 100 ml. of oxygen-free 50% aqueous ethanol.[10] The mixture was heated to about 70° and 1.3 g. of linoleic acid was added. The solution immediately became opalescent and turned milky on cooling to room temperature. After 4 hours of stirring, the crystalline solids were centrifuged out and dried, eventually over P_2O_5 at 0.5 mm. pressure. The yield was 7.7 g. Titration of two aliquots of the crude product showed a linoleic acid content of 7.28 and 7.37%, respectively. The titration of dextrin fatty acid compounds was carried out hot in 50% aqueous ethanol using 0.05 *N* KOH and phenolphthalein indicator. The accuracy of the method was tested with solutions of pure dextrin and model

(1) Hormel Institute publication no. 121. This investigation was supported by a grant from the United States Atomic Energy Commission.

(2) H. Schlenk and R. T. Holman, *Science*, **112**, 19 (1950).

(3) H. Schlenk and R. T. Holman, THIS JOURNAL, **72**, 5001 (1950).

(4) R. T. Holman and Siret Ener, *J. Nutrition*, **53**, 461 (1954).

(5) T. Sollman, "A Manual of Pharmacology," 6th ed., Saunders, Philadelphia, Penna., 1943, p. 912.

(6) Reviews on inclusion compounds: W. Schlenk, *Fortschr. Chem. Forsch.*, **2**, 92 (1951); F. Cramer, "Einschlussverbindungen," Springer-Verlag, Berlin, 1954.

(7) D. French, M. L. Levine, J. H. Pazur and E. Norberg, THIS JOURNAL, **71**, 353 (1949).

(8) K. Freudenberg, E. Plankenhorn and H. Knauber, *Ann.*, **558**, 1 (1947).

(9) H. Wieland and H. Sorge, *Z. physiol. Chem.*, **97**, 14 (1916).

(10) Solvent mixtures are given in volume % throughout.

mixtures containing known amounts of stearic acid. An aliquot of the crude preparation was put in a Warburg vessel to measure the oxygen uptake (Fig. 1, curve I). Another sample was freed of excess linoleic acid by distillation. The substance was placed in a test-tube which was connected by a 3-way stopcock to a high vacuum pump. After rinsing with nitrogen, the tube was heated in a horizontal position in an aluminum block at 120–125°. Microdroplets of acid distilled during the first half-hour into the cool part of the test-tube. Only traces of acid condensed upon further heating. It was found, however, that several hours of heating was necessary to obtain a stable product. In this particular preparation, the heating period was prolonged to 9 hours. The solids were separated from the distilled acid by cutting the test-tube below the ring of condensate. The dextrin contained 6.9% linoleic acid (Fig. 1, curve II). For larger preparations, an Abderhalden drying apparatus, filled with tetrachloroethylene to provide a distilling temperature of 122°, was used instead of the aluminum block.

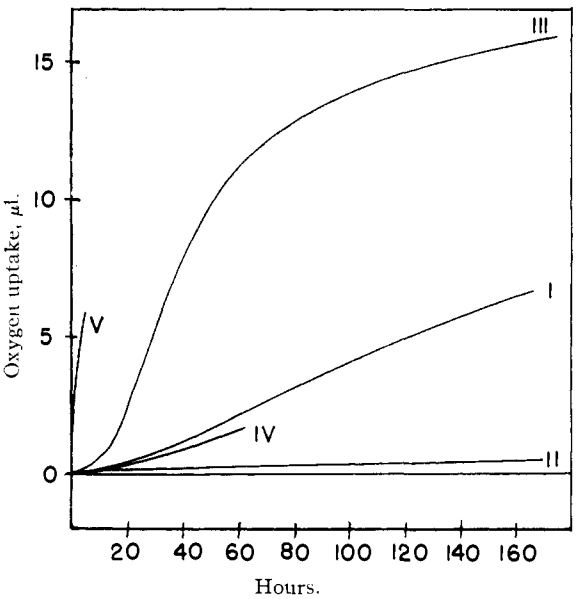

Fig. 1.—The rate of autoxidation of linoleic acid and of cinnamaldehyde, free and bound in β-dextrin: I, linoleic acid, 110 mg. in 1.5 g. of adduct, crude; II, linoleic acid, 117 mg. in 1.7 g. of adduct, purified by distillation; III, linoleic acid, 90 mg., control; IV, cinnamaldehyde, 105 mg. in 1 g. of adduct, purified by distillation; V, cinnamaldehyde, 120 mg., control.

The oxygen uptakes of the crude and purified preparations are compared to that of the free linoleic acid used for their preparation. Aliquots of the crude adduct also were washed with ethanol or trimethylpentane. The linoleic acid content was considerably decreased by the washing procedure but the rates of oxidation of the washed products were only slightly less than those of I.

The presence of unaltered linoleic acid in the complex was shown by dissolving 1.63 g. of purified adduct (II, after exposure to oxygen, containing 112 mg. of linoleic acid) in 100 ml. of hot 50% aqueous ethanol. The solution was extracted twice with 50-ml. portions of hot trimethylpentane and the extract dried with anhydrous Na_2SO_4. Titration of an aliquot of the trimethylpentane solution indicated complete recovery of linoleic acid. The trimethylpentane was removed from the remainder of the solution and the residual oil was brominated in low boiling petroleum ether (Skellysolve F).[11] White crystals (75 mg.) were obtained, which melted at 114° after slowly softening from 100° on. They were dissolved in a few drops of warm ether and reprecipitated by addition of Skellysolve F, yielding 47 mg. of pure tetrabromostearic acid, m.p. 115–116.5°.

(11) J. W. McCutcheon, *Org. Syntheses*, **22**, 76 (1942).

β-Dextrin–Linolenic Acid.—β-Dextrin (1.6 g.) and linolenic acid (0.32 g.) were treated as already described in 20 ml. of aqueous ethanol. The solids were isolated and heated for 17 hours at 122° (0.5 mm. pressure). Two 0.7-g. portions of the residue, each containing 67 mg. of linolenic acid, were exposed to pure oxygen in a Warburg apparatus. One of the Warburg flasks was kept dry as usual (test a), the other contained 0.5 ml. of water in the side arm to humidify the atmosphere (test b). Eventually hexabromostearic acid of the correct m.p. was obtained from the compound used in test (a) following the procedure described above for identifying linoleic acid.

β-Dextrin–methyl linolenate was similarly prepared. After purification by distillation, its content of linolenate was 10.8% (test c, 1 g. adduct containing 108 mg. ester). The amount of ester bound in the β-dextrin was determined by a modification of the procedure of Marcali and Rieman,[12] using 50% aqueous ethanol as saponification medium. Saponification of the homogeneous mixture is complete after one hour of heating over an aluminum block at 145–150° under smooth reflux.

The oxygen uptakes of the samples after 50 hours at 37° were: test a, 630 μl.; test b, 655 μl.; test c, 180 μl.; linolenic acid (control, 70 mg.), 12,700 μl.

β-Dextrin–Cinnamaldehyde.—β-Dextrin (5.0 g.) was dissolved in 100 ml. of water and 0.9 g. of cinnamaldehyde was added. After shaking for 16 hours at room temperature, droplets of free aldehyde could still be detected admixed with the crystals. The solids were isolated in the usual manner and heated to 100–140° at 0.5 mm. pressure for 3 hours. The residue had the flavor and taste of cinnamaldehyde. The rates of autoxidation of this product and of the free aldehyde are shown in Fig. 1, curves IV and V.

The aldehyde included in the dextrin was measured gravimetrically in the form of its 2,4-dinitrophenylhydrazone, which melts at 258–259° after recrystallization from acetic acid. The identity was ascertained by comparison with an authentic preparation. Glucose dinitrophenyl osazone having a similar m.p.[13] crystallizes quite differently and does not form under the conditions used here. The analytical data for the adduct are: 10.5% (9.6%), cinnamaldehyde; 0.3% (1.3%), cinnamic acid. The figures in parentheses give the percentages after the autoxidation test.

α-Dextrin–Linoleic Acid.—α-Dextrin (2.0 g.) was dissolved in 15 ml. of oxygen-free water and mixed with linoleic acid dissolved in 15 ml. of ethanol. After warming to 70° complex formation was allowed to take place for 4 hours at room temperature. The crystals were isolated by centrifugation and dried. Part of this was heated to 130–150° for 16 hours at 0.5 mm. pressure to remove excess linoleic acid (test a). The other portion of the crude preparation was suspended in approximately 10 ml. of ethanol, filtered and dried *in vacuo* over P_2O_5 (test b).

The oxygen uptakes of the two fractions were measured under standard conditions for 40 hours. After this period, values were: test a, 115 μl.; test b, 760 μl.; linoleic acid (control, 70 mg.) 7860 μl.

Deoxycholic Acid–Linolenic Acid.—The general procedure for preparing choleic acids has been described by Wieland and Sorge[9] and by Rheinboldt.[14] In agreement with the observation made with urea[2,3] and thiourea,[15] however, it also was found that unsaturated molecules form crystalline adducts with deoxycholic acid only in high concentration or at low temperature. Deoxycholic acid (6.0 g.) was dissolved in 20 ml. of absolute ethanol and 0.55 g. of linolenic acid in 5 ml. of ethanol was added. Crystallization was complete after 16 hours at −5 to −10°. The isolated crystals after drying contained 8.3% linolenic acid. The autoxidation curve of this product is shown in Fig. 2.

The fatty acid content in choleic acids was determined by simplifying the method of Wieland and Sorge.[9] The complex was refluxed with eight times its weight of xylene for 1 hour and the xylene adduct of deoxycholic acid was filtered off and washed with benzene. The combined xylene and benzene solutions were evaporated, and the oily residue was extracted with Skellysolve C and filtered. The fatty acids were titrated after removal of the solvent.

(12) K. Marcali and W. Rieman, *Ind. Eng. Chem., Anal. Ed.*, **18**, 144 (1946).

(13) A. L. Lehninger, *J. Biol. Chem.*, **149**, 43 (1943).

(14) H. Rheinboldt, *Ann.*, **451**, 256 (1927); **473**, 249 (1929).

(15) W. Schlenk, *ibid.*, **573**, 150 (1951).

Deoxycholic Acid–Vitamin A Palmitate.—Since small scale preparations of pure adducts with vitamin A alcohol or acetate met with difficulties, the palmitate of vitamin A was chosen for this experiment. The long saturated chain of palmitic acid provided a good anchor aiding complex formation. Deoxycholic acid (1.0 g.) and vitamin A palmitate (0.1 g.) were dissolved in 4 ml. of hot absolute ethanol. The mixture was cooled to room temperature, and eventually kept at −3° for 12 hours. The light yellow crystals, after filtering, were dried in high vacuum. They contained 10.8% vitamin A palmitate. The autoxidation of this adduct is compared to that of free vitamin A palmitate in Fig. 2, curves III and IV.

The vitamin assay was carried out spectrophotometrically in ethanol at 325 mμ.[16] From model mixtures it was ascertained that the absorption curve is not influenced by the presence of deoxycholic acid.

Results and Discussion

All complexing agents so far tested, *i.e.*, urea,[2,3] α- and β-dextrins and deoxycholic acid, lower greatly the autoxidizability of the moiety included. In addition to fatty acids having non-conjugated *cis* double bonds, vitamin A palmitate having a conjugated system and cinnamaldehyde could be protected. In their fundamental publication on choleic acids, Wieland and Sorge[9] state that benzaldehyde in combination with deoxycholic acid is stable in air. Little attention has been given to Wieland's observation in the intervening decades when the chemistry and crystallography of choleic acids was studied extensively.[4,17] The quantitative results presented here and previous studies on urea adducts suggest that all autoxidizable systems are protected when included.

Of the inclusion compounds used in this study, the choleic acids are the best known. Their stoichiometry is well explained by comparison of the chain length of the guest molecule with the chain length of the channel built by the corresponding number of deoxycholic acid molecules. However, for fatty acids or their derivatives having chains longer than 20 atoms the rules are not valid. The molar ratio of linolenic acid and deoxycholic acid is 1:7.8 in our stable product. It is in good agreement with the value of 1:8 for stearocholeic acid. So for the C_{18} series unsaturation does not change the stoichiometry of the adduct. The tendency to form the adduct is markedly lowered by unsaturation, and the use of deoxycholic acid for fractionations similar to those performed with urea seems feasible. In numerous preparations of the vitamin choleic acid the content of vitamin A palmitate varied between the extreme values 9.2 and 11.7%, *i.e.*, molar ratios of 1:10.1 and 1:13.2. All these preparations showed very good resistance to autoxidation, although the stabilization was not always as complete as in the example given in the Experimental part. It was not possible to determine the "maximum load" deoxycholic acid will take into its structure. Any excess deoxycholic acid, of course, does not interfere with the stabilization.

The phenomenon of gas being released from inclusion compounds when tested under oxygen at 37° already was encountered when urea compounds were studied, although no special mention of it was made then (Fig. 3, in ref. 3). It is conceivable that a substance exerting strong forces upon large guest molecules has pronounced adsorptive power toward gas molecules too. One might even consider the spongy structure of the host as a material that is "surface all the way through." Still it is uncertain if binding of gases takes place on the surface or inside the crystals.

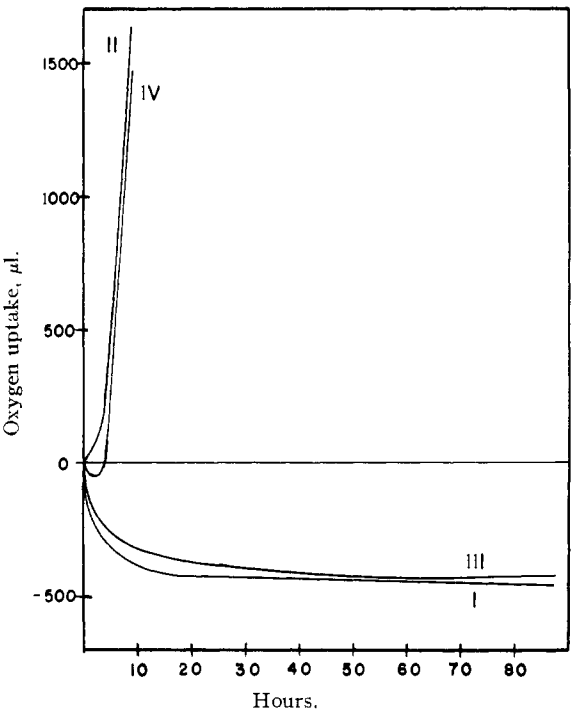

Fig. 2.—The rate of autoxidation of linolenic acid and of vitamin A palmitate, free and bound in deoxycholic acid: I, linolenic acid, 87 mg. in 1.05 g. of adduct, purified by distillation; II, linolenic acid, 70 mg., control; III, vitamin A palmitate, 21.6 mg. in 200 mg. of adduct; IV, vitamin A palmitate, 25 mg., control.

Difficulties were found in preparing stable adducts of the cyclic dextrins. Preparations of adducts of smaller molecules from aqueous dextrin solutions have been described.[18–20] The products we obtained from C_{18}-acids or cinnamaldehyde by such procedures were not stable against autoxidation. Due to the low solubility of these materials, the presence of water is undesirable for the preparation of pure complexes. Water, however, seems to be a necessary component for the reaction as the following experiments indicate. No reaction takes place when β-dextrin is heated and shaken with myristic acid in methanol; dry α- and β-dextrins do not react with dry vapors of trichloroethylene or bromobenzene, both known to be typical adduct formers; in contrast to this, when water is present either as vapor in the atmosphere or in the form of crystalline hydrates of the dextrins, weight increases up to 26% are found and the products show very strong halogen tests.

A satisfactory solvent for both dextrin and lipid could not be found and, in a compromise, 50% aque-

(16) P. György, "Vitamin Methods," Academic Press, Inc., New York, N. Y., 1950, Vol. 1, p. 14.

(17) Y. Go and O. Kratky, *Z. physik. Chem.*, **B26**, 439 (1934); O. Kratky and G. Giacomello, *Monatsh.*, **69**, 427 (1936); V. Caglioti and G. Giacomello, *Gazz. chim. ital.*, **69**, 245 (1939).

(18) K. Freudenberg and F. Cramer, *Ber.*, **83**, 296 (1950).

(19) F. Cramer, *ibid.*, **84**, 851 (1951).

(20) F. Cramer, *Ann.*, **579**, 17 (1953).

ous ethanol was used. The solubility of β-dextrin in such a mixture is about equal to that in water. Enough water is present to enable adduct formation and at the same time the solubility of the lipid is slightly increased to facilitate the reaction. Oil droplets still were detectable, admixed with the crystalline adducts, even when a large excess of dextrin was used. Implying that stabilization indicates inclusion and that autoxidation indicates non-included contamination, we never could obtain pure (stabilized) adducts directly from the mixture. Such crude preparations show high rates of autoxidation for which curve I in Fig. 1 is typical. Washing with ethanol or trimethylpentane was found unreliable for removal of excess lipid. By such treatment, the rate of autoxidation is lowered but at the same time part of the included moiety also is extracted. Reproducible stabilization can be achieved by distilling off the contaminating material although the distillation does not come to a complete halt at that point. Therefore these products do not represent the maximum molar ratio host:guest. In the stable adducts the ratios are between 2.3:1 and 3.6:1 for C_{18}-derivatives, for cinnamaldehyde it is close to 1:1, when the values are calculated for the dry preparations. The heat treatment renders the products very hygroscopic.

The adducts of dextrins do not release gas when kept under dry oxygen. In the presence of water considerable amounts of gas are given off in particular from samples dehydrated by heat. Most likely this is in conjunction with a rehydration process. Sometimes hydrated products exhibit stabilities even higher than identical samples when kept dry. This, however, is not a general rule.

Unsaturated fatty acids are the most convenient material to test for inclusion protection against oxygen. Some autoxidation of the β-dextrin–cinnamaldehyde adduct (Fig. 1, IV) is to be expected since it has a demonstrable vapor tension of aldehyde. Stabilization of vitamin A palmitate is very satisfactory in deoxycholic acid (Fig. 2, III). In β-dextrin the autoxidation of vitamin A alcohol and acetate could be cut down to $^1/_{10}$ to $^1/_{20}$ of the original. These results are not yet satisfactorily reproducible and preparative conditions must still be refined.

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AUSTIN, MINNESOTA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Proximity Effects. I. 6-Aminocyclodecanol and 11-Azabicyclo[4.4.1]-1-undecene from 6-Aminocyclodecanone

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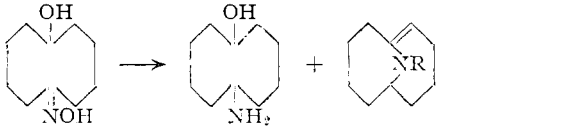
Reduction of 6-hydroxycyclodecanone oxime (I) with sodium and *n*-butyl alcohol has been found to yield a mixture of 6-aminocyclodecanol (II, 72%) and 11-azabicyclo[4.4.1]-1-undecene (III, 24%). Formation of the unsaturated secondary amine III is explained by the spatial proximity of the secondary alcohol and oximino groups of I. An intramolecular oxidation–reduction of the Meerwein–Ponndorf–Verley type is believed to result in partial conversion of I to 6-aminocyclodecanone (IV), which is converted to III by dehydration. The unsaturated amine III is of interest because it contains a double bond at the bridgehead of a heterocyclic [4.4.1]ring system. Evidence for the structure of III is provided by its ultraviolet and infrared spectra, by conversion to a benzenesulfonamide and phenylthiourea, by hydrolysis with hydrochloric acid to 6-aminocyclodecanone (IV, reconverted to III on heating), and by quantitative reduction to the saturated amine V. The structure of V was verified by degradation by the Hofmann exhaustive methylation procedure, and by an independent synthesis from 6-aminocyclodecanol.

This paper describes the reduction of 6-hydroxycyclodecanone oxime (I) to 6-aminocyclodecanol (II), required as an intermediate in the synthesis of *trans*-5-cyclodecen-1-ol.[3] When the oxime I was reduced with sodium and *n*-butyl alcohol, the unsaturated secondary amine 11-azabicyclo[4.4.1]-1-undecene (III) was formed as an unexpected by-product. Formation of the amine III from the oxime I and other unusual reactions described in this and the following paper are ascribed to "proximity effects," *i.e.*, to the fact that atoms located on opposite sides of medium-sized rings (ten-membered in this instance) are brought into close proximity by the geometric conformations of the rings.



I II III, R = H
VI, R = $SO_2C_6H_5$
VII, R = $C(NHC_6H_5)=S$

6-Hydroxycyclodecanone was prepared by the procedure described by Criegee,[4] as subsequently adapted to larger scale preparations,[5] with modifications in the procedure for oxidizing decalin and purifying *trans*-9-decalylhydroperoxide that are described in the Experimental part. Reaction of 6-hydroxycyclodecanone with hydroxylamine hydrochloride in pyridine and absolute ethanol formed the oxime I in 86–90% yield.

Hydrogenation of the oxime I in ethanol in the presence of W-2 Raney nickel[6] at room temperature

(1) American Cyanamid Co. Fellow, 1953-1954.

(2) Arthur D. Little Fellow, 1948–1949.

(3) A. C. Cope, R. J. Cotter and G. G. Roller, THIS JOURNAL, **77**, 3594 (1955).

(4) R. Criegee, *Ber.*, **77B**, 22, 722 (1944); R. Criegee and H. Dietrich, *Ann.*, **560**, 135 (1948); R. Criegee and W. Schnonenberg, *ibid.*, **560**, 141 (1948).

(5) A. C. Cope and G. Holzman, THIS JOURNAL, **72**, 3062 (1950).

(6) R. Mozingo, *Org. Syntheses*, **21**, 15 (1941).