Two grams of the ester was converted into the amide by treatment with sodium amide as described above in (c). Evaporation of the ether left an oily residue which failed to crystallize. The residue was heated to boiling in a short test-tube and approximately one-half of it distilled off. The remaining material solidified on cooling and melted at 43–44°. Methylethylpropylacetamide has been reported to melt at 46°[20] and at 42–43°.[21]

Alkylation of Ethyl Isovalerate with Ethyl Iodide.—To an ether solution of sodium triphenylmethyl[9] containing 0.205 mole of the base was added with shaking 26.6 g. (0.205 mole) of ethyl isovalerate (Eastman). After two minutes, 32.0 g. (0.205 mole) of ethyl iodide (b. p. 72°) was added. The reaction vessel was stoppered, shaken and allowed to stand overnight. The reaction mixture was worked up as in the preceding experiment. The material remaining after the removal of ether was vacuum-distilled up to 150° at 15 mm. After two or three fractionations at atmospheric pressure through a twelve-inch Widmer column, the following fractions were obtained from the distillate: (1) up to 132°, 2.5 g., (2) 132–140°, 4.0 g., (3) 140–150°, 3.5 g., (4) 150–163°, 3.6 g., (5) 163–166°, 5.9 g. Vacuum distillation of the residue from the fractionation yielded 2.3 g. of material boiling at 118–122° at 15 mm. Fraction (2), boiling at 132–140°, probably consisted largely of recovered ethyl isovalerate (b. p. 135°); most of this fraction came over at 134–138°. The boiling point of fraction (5), 163–166°, is in agreement with the boiling point reported in the literature[22] for ethyl α-ethylisovalerate. The yield of this ester, 5.9 g., was 22% of the theoretical amount. A portion of the ester was converted into the amide by the method described above in (c). After one recrystallization from ligroin (90–120°) the amide melted at 135–135.5° in agreement with the melting point reported in the literature for α-ethylisovaleramide.[22] The fraction boiling at 118–122° at 15 mm. was redistilled at 32 mm. and boiled at 129–132° in agreement with the boiling point reported in the literature[23] for ethyl isovalerylisovalerate. The redistilled material was hydrolyzed[24] to di-isobutyl ketone by refluxing three hours with 25 cc. of glacial acetic acid containing 10% sulfuric acid and 10% water by volume. The hydrolysis mixture was kept cold and made alkaline with sodium hydroxide, then extracted three times with ether. The ether was distilled off and the crude ketone (1.9 g.) converted directly into the semicarbazone. The latter melted at 121–122° in agreement with the melting point reported in the literature.[23]

Attempted Alkylation of Ethyl Acetate.—The addition of 16 g. (0.18 mole) of ethyl acetate to a rapidly stirred solution containing an equivalent quantity of sodium triphenylmethyl[9] was followed in ten seconds by the addition of 31 g. (0.18 mole) of benzyl chloride. After allowing the mixture to stand overnight and working up essentially as described above in (a), only 3 to 4 g. of material, boiling at 100–200° at 15 mm., which may have contained ethyl hydrocinnamate (b. p. approximately 150° at this pressure) was obtained. Twenty-five grams of lower boiling material, presumably largely ethyl acetoacetate, was obtained.

Summary

The di-substituted esters, ethyl isobutyrate and ethyl methylethylacetate, have been successfully alkylated by first converting the esters into their sodium enolates by means of sodium triphenylmethyl, and treating the enolates with alkyl halides. Treated in a similar manner, ethyl isovalerate gave a fair yield of the ethylated product, but ethyl acetate failed to give an appreciable amount of the alkylated ester.

(20) Haller and Bauer, *Compt. rend.*, **148**, 130 (1909).
(21) Haller and Bauer, *Ann. chim.*, (9) **1**, 15 (1914).
(22) Crossley and LeSueur, *J. Chem. Soc.*, **77**, 94 (1900).
(23) Spielman and Schmidt, THIS JOURNAL, **59**, 2010 (1937).
(24) The method of hydrolysis described here has been used successfully by the authors for the conversion of several β-keto esters to their corresponding ketones.

DURHAM, N. C. RECEIVED JULY 3, 1940

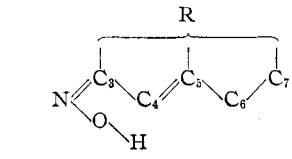
[A CONTRIBUTION FROM THE LABORATORY OF BIOLOGICAL CHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF BUFFALO]

Stereoisomeric Oximes of Cholestenone

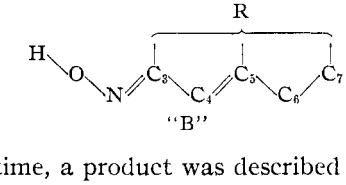
BY J. O. RALLS

Introduction

The isolation of the *syn* and *anti* oximes of an α, β unsaturated ketone was reported for the first time in 1931.[1] In 1938, the author suggested that some peculiarities observed during the reaction of cholestenoneoxime with iodine monobromide might be explained by means of the assumption that two stereoisomeric cholestenoneoximes existed, which, for want of better designations, were called "A" and "B."[2]



"A"



"B"

At that time, a product was described which had

(1) Blatt and Stone, THIS JOURNAL, **53**, 4135 (1931).
(2) Ralls, *ibid.*, **60**, 1748 (1938).

been obtained simply by recrystallizing cholestenoneoxime from glacial acetic acid. It contained the same amount of nitrogen and had the same ultraviolet absorption characteristics as did the cholestenoneoxime from which it was obtained. Hence it was not an isoxazoline. It melted at 65°, but was reconverted to the original oxime, m. p. 151.8°, when it was heated to a temperature above 85°. These facts led us to postulate that the compound was a stereoisomer of cholestenoneoxime "B." This paper presents confirmation of data obtained previously and new data which make the original postulate more valid.

Discussion

A considerable quantity of "A" (melting at 65°, resolidifying at 85–90°, and melting again at 151.8°) was prepared by recrystallizing cholestenoneoxime from glacial acetic acid. It had the same carbon, hydrogen, and nitrogen content and the same molecular weight as the original oxime.

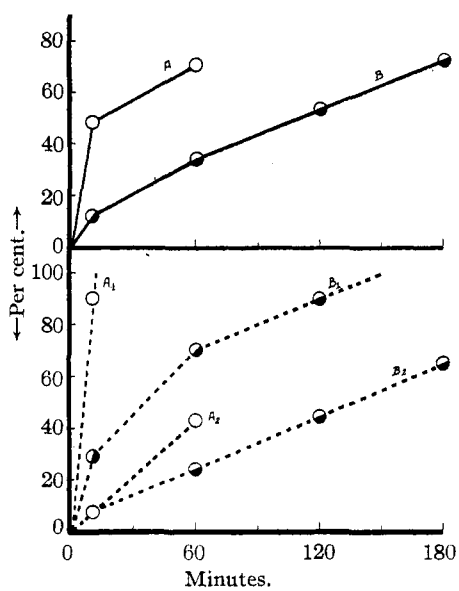


Fig. 1.—Relative rates of removal of bromine from brominated cholestenoneoximes by alcoholic potassium hydroxide (at 80–82°): O———O, Curve A, the % of the total halogen of brominated "A"; ◒———◒, Curve B, the % of the total halogen of brominated "B." Acting upon the reasonable assumption that the bromines are attached to carbon atoms 4 and 5, and utilizing the experience of others who found that the bromine at 5 is less firmly attached than is the bromine at 4, the following curves were calculated from those above: O----O, Curve A_1, calcd. % of the C_5 bromine liberated from brominated "A"; O----O, Curve A_2, calcd. % of the C_4 bromine liberated from brominated "A"; ◒----◒, Curve B_1, calcd. % of the C_5 bromine liberated from brominated "B"; ◒----◒, Curve B_2, calcd. % of the C_4 bromine liberated from brominated "B" (a mixture).

The reconversion of "A" to "B" could be effected not only by means of heat, but also by the mere solution of "A" in benzene or carbon tetrachloride (Table I). In the case of the use of heat, the change was not due to a loss of water of hydration or of solvent of crystallization. On the other hand, the conversion of "B" to "A" could be effected by recrystallization from propionic or *n*-butyric acid, as well as from glacial acetic acid (Table I), while formic acid had no demonstrable effect upon either "A" or "B." The interconversion of stereoisomeric ketoximes is by no means new to the literature, for formic acid, and alcoholic alkali,[1] and dry hydrogen chloride, heat, and ultraviolet light[3] have been known to induce such changes.

It is realized that, up to the present, no absolute evidence that our two compounds are *syn* and *anti* isomers has been offered. Because the carbonyl group in cholestenone is part of a complex ring structure and not in an aliphatic chain, we could not use the Beckmann rearrangement to prove our point and had, therefore, to look to other means. It seemed that ring closure studies might supply the needed evidence, since, according to Blatt,[4] "by far the greater part of the chemical evidence of oxime configuration has resulted from the study of ring closure of appropriately substituted ketoximes." Cathcart and Meyer[5] discovered that certain ortho substituted benzophenoximes lost halogen acid upon being treated with alcoholic alkali. Later Meisenheimer, *et al.*,[6] pointed out that this loss of halogen acid with the consequent formation of indoxazene (isoxazoline) is sufficient evidence of the *syn* configuration of the oxime being studied. For these reasons, we brominated our two compounds: "A" in glacial acetic acid, and "B" in carbon tetrachloride. The bromo derivative of "A" was definitely crystalline (m. p. 96°) and lost halogen acid very readily when treated with alcoholic potassium hydroxide (Fig. 1). In fact, it lost halogen acid spontaneously while standing in the dry state. This decomposition product melted at 87° and still contained one atom of bromine. On the

(3) Sidgwick, "The Organic Chemistry of Nitrogen," Clarendon Press, Oxford, 1937, pp. 182, 192.

(4) Blatt, *Chem. Rev.*, **12**, 225, 226 (1933).

(5) Cathcart and Meyer, *Ber.*, **25**, 1498, 3291 (1892).

(6) Meisenheimer and Meis, *ibid.*, **57**, 289 (1924); Meisenheimer, Zimmermann and Kummer, *Ann.*, **446**, 205 (1926); Meisenheimer, Hanson and Wächterowitz, *J. prakt. Chem.*, (2) **119**. 315 (1928).

other hand, the bromo derivative of "B" was not definitely crystalline and was apparently either a mixture of mono- and dibromo compounds, or a mixture of a dibromo compound and one containing no halogen. This mixture (m. p. 123–125°) did not lose halogen acid so rapidly, even when treated with alcoholic potassium hydroxide at 82° (Fig. 1). The behavior of the two brominated compounds suggested that, in "A," the hydroxyl was indeed *syn* while, in "B," it was *anti* with respect to the bromine saturated double bond. If such was actually the case, it seemed that the ready loss of halogen acid from brominated "A" should have been attended by the formation of an isoxazoline, which, in turn should not react with acetic anhydride. The product derived from brominated "A" did not acetylate. It appeared, therefore, that the *syn* configuration of "A" was definitely established. However, this certainty was somewhat vitiated by the fact that brominated "B" did not acetylate either. It must be admitted that this last result was not clean cut inasmuch as the attempted reaction was accompanied by considerable decomposition (Table II).

The failure of the product from brominated "A" to consume acetic anhydride was expected, but that failure on the part of brominated "B" was not expected, for even with marked decomposition some one cleavage product should have still carried an hydroxylimino group (nitrile formation is not likely when the carbon bearing the N-OH is part of a ring). Apparently, the only explanation for the results obtained was that brominated "B" rearranged to brominated "A" which then formed an indoxazene that could not react with acetic anhydride. Blatt[1] has reported that, during the bromination of *anti*-benzal-*p*-bromoacetophenoxime, a rearrangement to the *syn* form took

TABLE I

THE MELTING POINTS OF SAMPLES OF CHOLESTENONEOXIME RECRYSTALLIZED OR RECOVERED[a] FROM VARIOUS SOLVENTS

Initial material, cholestenoneoxime		"B," m. p. 151.80	"A," m. p. 650
Melting points, °C., of crystals from	EtOH	151.8	65
	Benzene[a]	151.8	151.8
	CCl_4[a]	151.8	151.8
	HCOOH	151.8	65
	Gl. AcOH	65	65
	Propionic acid	65	65
	n-Butyric acid	65	65

[a] When crystallization could not be effected, the solvent was evaporated *in vacuo* at a low temperature and the melting point of the residue was determined.

TABLE II

ANALYTICAL RESULTS OF QUANTITATIVE ACETYLATIONS

[a]Milliequivalents × 10

Material	Sample	Acid equiv. of acetic anhydride	Alkali equiv. added	Acid equiv., back titration	HBr liberated	Excess acetic anhydride	Consumed acetic anhydride	Expected[b]
Blank	0.00	27.06	29.23	2.17	0.00	27.06	0.00	0.00
Blank	.00	27.06	29.23	2.21	.00	27.02	.04	.00
Blank	.00	27.06	29.23	2.13	.00	27.10	− .04	.00
3-Phenyl-5-*p*-bromo-	1.00	↑	↑	2.24	↑	26.99	.07	.00
phenylisoxazoline	1.00			2.17		27.06	.00	.00
	0.96			2.18		27.05	.01	.00
syn-Styryl-*p*-bromo-	1.00			3.15		26.08	.98	1.00
phenylketoxime	1.00			3.18	↓	26.05	1.01	1.00
Cholestenoneoxime	1.00			3.23	.00	26.00	1.06	1.00
	1.00			3.17	.00	26.06	1.00	1.00
	1.00			3.16	.00	26.07	0.99	1.00
Derivative[c] from	1.00			1.78	.39	27.06	.00	0.00
brominated choles-	1.00			1.85	.30	27.08	− .02	.00
tenoneoxime "A"	1.00			1.77	.40	27.06	.00	.00
Derivative[d] of bromi-	1.00	27.06	29.23	1.45	.76	27.02	.04	1.00
nated cholestenone-	1.00	27.06	29.23	1.37	.82	27.04	.02	1.00
oxime "B"	1.00	27.06	29.23	1.32	.88	27.03	.03	1.00

[a] The figures in the "sample" column are weights of material in milligrams divided by one-tenth of the molecular weight. The figures in the other columns are cc. × normality × ten. [b] Result expected or hoped for. [c] The product resulting from the spontaneous cleavage of halogen acid from brominated cholestenoneoxime "A" (see experimental). [d] The product derived by the treatment of brominated cholestenoneoxime "B" with alcoholic potassium hydroxide (See experimental).

place. But because we obtained two distinct bromo derivatives, it was not believed that the suggested change occurred during their preparation. If it did occur at all, it must have been during the attempted acetylation, at which time a marked liberation of halogen acid was actually noted (Table II).

Inasmuch as Blatt[1] had been successful in distinguishing between the *syn* and *anti* forms of benzal-*p*-bromoacetophenoxime through the preparation of their acetates, it was decided to prepare the acetates of cholestenoneoximes "A" and "B." Instead of definitely crystalline compounds, we obtained two glasses. These differed in their indices of refraction (Table III). The glass that

TABLE III

THE REFRACTIVE INDICES OF CHOLESTENONEOXIME ACETATES PREPARED UNDER VARIOUS CONDITIONS

Conditions of reaction			*n* at various temperatures			
Solvent	Temp., °C.	Time, hr.	60°	55°	50°	45°
Dioxane	25	72	1.5182	1.5194	1.5209	1.5229
	80–85	3	1.5221	1.5232	1.5247	1.5267
Gl. acetic acid	25	72	1.5221	1.5232	1.5247	1.5267
	80–85	3	1.5221	1.5232	1.5247	1.5267[a]

[a] The values were obtained with a "Spencer" Abbe refractometer and are correct to within ±0.0002.

resulted from the acetylation in glacial acetic acid, or in dioxane at 80–85°, had the higher index. (As stated before, glacial acetic acid favored the formation of "A.") It is known that, of *cis* and *trans* isomers, the former generally has the lower melting point[7,8] and the higher density[7–12] and, moreover, that the *cis* compound usually has a slightly higher index of refraction and, consequently, lower specific and molecular refractions.[9–12] These rules apply to *syn* (*cis*) and *anti* (*trans*) isomeric oximes also.[13] An experimental error may have been responsible for the higher index of refraction of the "glass" derived from "A," but if this were the case, the error was repeated in determinations of the index of refraction of solutions of "A" and of "B," for the 1.22 + % solution of "A" in 95% alcohol had a higher index of refraction, a higher density, and lower specific and molecular refractions than a similar solution of "B." Since it is not probable that this same error was repeated, it is felt that the differences in the determined indices were real.

(7) Anschütz, *Ber.*, **11**, 1644 (1878).
(8) Michael, THIS JOURNAL, **40**, 1674 (1918).
(9) Brühl, *J. prakt. Chem.*, **50**, 152 (1894).
(10) Skita, *Ber.*, **53**, 1792 (1920); *ibid.*, **55**, 144 (1922); *Ann.*, **427**, 255 (1922); *ibid.*, **431**, 1–15 (1923); *Ber.*, **56**, 1014 (1923).
(11) K. v. Auwers, *Ann.*, **432**, 94 (1923).
(12) Langseth, *Z. physik. Chem.*, **118**, 49 (1925).
(13) K. v. Auwers and Ottens, *Ber.*, **57**, 437, 489 (1924).

The evidence cited above left no doubt in mind that "A" was an isomer of "B," but the possibility that the former compound was $\Delta^{5,6}$, while the latter was $\Delta^{4,5}$, had not been excluded. However, $\Delta^{5,6}$-cholestenoneoxime has been reported[14] to have a melting point of 188°. Therefore, "A" could not have been that compound. Having excluded this last possibility, we concluded that "A" was *syn*-3-hydroxylimino-cholesten-4,5, whereas "B" was *anti*-3-hydroxylimino-cholesten-4,5, in which the 4,5 double bond is the reference point.

Materials.—Cholesterol,[15] m. p. 147.5°, purified through the dibromide.[16,17] Cholestenone, $\Delta^{4,5}$, m. p. 81°, prepared according to Windaus[18] and Schoenheimer.[19] Cholestenoneoxime, m. p. 151.8°, prepared according to Diels and Abderhalden.[20]

Experimental

The Action of Glacial Acetic Acid upon Cholestenoneoxime.—Each of four 1.2-g. portions of cholestenoneoxime "B" was dissolved in a separate 5-cc. portion of glacial acetic acid. The material dissolved readily, but, within three minutes, a mass of crystals separated from each solution. These crystals, "A," were filtered, washed with alcohol, and dried by suction. The average yield was 84% of the original sample. Samples of each batch of crystals melted at 65°, resolidified at 85–90°, and melted again at 151.8° (the latter is the melting point of the original oxime). No sample of cholestenoneoxime, directly prepared and recrystallized from alcohol alone, ever displayed similar peculiarities in melting.

Weighed portions of "A," when heated to 120°, exhibited a loss in weight of 1.70–1.85%. If this decrease in weight were due to loss of solvent of crystallization, it should have been: for 1 CH_3COOH, 13.0%; for 1 C_2H_5OH, 10.3%; for 1 H_2O, 4.2%; and for $^1/_2$ H_2O, 2.2%.

Some "A" was dried over phosphorus pentoxide *in vacuo* at 45°, after which it also exhibited the same peculiar melting behavior. These especially dried crystals lost only 0.03% of their weight upon being heated to 120°. The melting phenomenon apparently was not due to a change from a "hydrated" to a dry form of cholestenoneoxime.

Anal. Found for the treated oxime, "A": C, 81.1; H, 11.38; N, 3.51. Found for the untreated oxime, "B": C, 81.0; H, 11.40; N, 3.50. Calcd. for $C_{27}H_{45}ON$ (oxime): C, 81.12; H, 11.35; and N, 3.50. Calcd. for $C_{29}H_{47}O_2N$ (oxime acetate): C, 78.84; H, 10.73; and N, 3.17.

The Determination of the Molecular Weight of Rearranged Cholestenoneoxime (cryoscopically).

(14) Butenandt and Schmidt-Thomé, *ibid.*, **69B**, 882–888 (1936).
(15) The author is grateful to the Wilson Laboratories for their generous gift of a quantity of cholesterol.
(16) Koch, Koch, and Kraus-Ragins, *J. Biol. Chem.*, **85**, 141 (1929–1930).
(17) Hess and Anderson, *ibid.*, **74**, 651 (1927).
(18) Windaus, *Ber.*, **39**, 518 (1906).
(19) Schoenheimer, *J. Biol. Chem.*, **110**, 461 (1935).
(20) Diels and Abderhalden, *Ber.*, **37**, 3101 (1904).

Benzene, g.	Oxime, g.	Δ°	Mol. wt. found
17.54	0.046	0.034	397.4 ± 10
17.54	.097	.071	398.0 ± 5
Glacial acetic acid, g.			
20.90	0.1012	0.043	439+
20.90	.067	.033	378
		Av.	409

A considerable degree of difficulty was experienced in the determinations involving the use of acetic acid, but no difficulty was met in those involving benzene.

The Effect of Benzene upon Rearranged Cholestenoneoxime "A."—The solutions in benzene used in the molecular weight determinations were allowed to evaporate at room temperature. The residues were washed, by means of a stream of alcohol, onto a small Büchner funnel and were dried by suction. These materials melted at 151°. The "A" compound had reverted to "B."

The Effect of Carbon Tetrachloride and of Glacial Acetic Acid upon Cholestenoneoxime "A."—Thirty milligrams of "A" was dissolved in 6 cc. of carbon tetrachloride. The solution was allowed to evaporate spontaneously. The residue melted at 151°. Another such solution was evaporated *in vacuo* at a low temperature. Its residue also melted at 151°. Reversion of "A" to "B" had occurred in the carbon tetrachloride.

Thirty milligrams of "A" was dissolved in a few cc. of glacial acetic acid. The solution was carefully evaporated *in vacuo*. The residue melted at 65°, solidified at 90°, and remelted at 151°. It seemed safe to assume that, in a solution of "A" in glacial acetic acid, the compound remains in that form.

The Effect of Formic Acid upon Cholestenoneoximes "A" and "B."—When 1 g. of cholestenoneoxime "B" (m. p. 151.8°) was treated with 5 cc. of formic acid (99.5 + %), it dissolved immediately. No crystals appeared when the solution was chilled, but some were formed when either water or alcohol was added. The dried crystals melted at 151°. Hence, the original substance was recovered.

When cholestenoneoxime "A" was likewise treated with formic acid, the product obtained melted at 65°, solidified at 85–90°, and remelted at 151.8°. Because of these results, it appeared that formic acid was a solvent in which either form of cholestenoneoxime could exist.

The Effect of Propionic Acid upon Cholestenoneoxime "B."—The solution of 1 g. of cholestenoneoxime in 5 cc. of propionic acid was effected by vigorous stirring. When this solution was chilled, crystals appeared. They were filtered off and washed thoroughly with alcohol. The product (and that from the chilled alcohol washings) melted at 65°, solidified at 85°, and remelted at 151°. Rearrangement of the cholestenoneoxime had been induced by the propionic acid.

The Action of *n*-Butyric Acid upon Cholestenoneoxime "B."—Gentle heating was required to dissolve 1 g. of cholestenoneoxime in 5 cc. of *n*-butyric acid. When the solution was cooled, a relatively large quantity of crystals separated. These were washed repeatedly with cold alcohol until the odor of butyric acid was no longer noticeable. The dry crystals behaved, upon melting, as did those from glacial acetic and propionic acids. "B" had been converted to "A."

The Preparation of a Bromo Derivative of Cholestenoneoxime "A."—Three grams of cholestenoneoxime "A" was dissolved in 60 cc. of glacial acetic acid and 2.4 g. of bromine in 60 cc. of glacial acetic acid was added. After a few minutes, a precipitate of fine white needles separated. These were filtered, washed, and dried with suction. They contained halogen and melted at 96°.

When weighed samples of the crystals were treated with alcoholic potassium hydroxide, at 82°, they lost halogen acid equivalent to 13.7 and 20.2% of their weight in ten and sixty minutes, respectively. The liberated halogen acid was determined by a modified Volhard procedure.

The remainder of the product, about 0.8 g., was stored in a tightly stoppered vial. Two days later, it was found to have decomposed with the liberation of a relatively large amount of halogen acid. The residue was triturated with water, filtered, and dried with suction. The product melted at 87°. As determined by the Willard–Thompson[21] procedure, it still contained 16.2% of bromine. The calculated per cent. of bromine in $C_{27}H_{45}BrON$ is 16.7 and hence it appeared that the product was a monobromo derivative.

The Preparation of a Bromo Derivative of Cholestenoneoxime "B."—Three grams of cholestenoneoxime was dissolved in 60 cc. of carbon tetrachloride and 2.4 g. of bromine in 60 cc. of carbon tetrachloride was added. No product separated out. The solution was allowed to stand overnight at room temperature, after which it was taken to dryness *in vacuo*. The residue was dissolved in ether. When alcohol was added to the solution, a precipitate formed which was poured onto a suction filter and partially dried. It was then redissolved in ether and reprecipitated with alcohol. The dry product did not decompose on standing. It melted at 123–126°, with decomposition, and contained 20.6% bromine. Since $C_{27}H_{45}BrON$ contains 16.7 and $C_{27}H_{45}Br_2ON$ contains 28.3% bromine, it appeared that the product was a mixture of about 66% mono- and 34% dibromo derivatives of cholestenoneoxime, or 73% dibromo and 27% unhalogenated material.

When weighed portions of the mixture were treated with alcoholic potassium hydroxide at 82°, they lost halogen acid equivalent to 7.1, 11.0, and 15% of their weights in sixty, one hundred and twenty, and one hundred and eighty minutes, respectively.

Attempted Differentiation between Monobromocholestan-3,5-isoxazoline (?) and Bromocholestenoneoxime, by Acetylation.—A 1:12.5 dilution of acetic anhydride in 1,4-dioxane was prepared. A small all-glass reaction vessel with a condenser extending well down into the reaction chamber was made from Pyrex glass. One-tenth milliequivalent of the material under examination was weighed into a *small* glass dish which was then placed in the bottom of the above vessel; 1.5 cc. of the acetic anhydride solution was added. The condenser glass stopper was inserted and water was started through it. The whole apparatus was suspended in a water-bath at 85°.

After two hours, the apparatus was removed and, while it cooled, distilled water was allowed to trickle down into the chamber past the slightly loosened stopper. When the vessel was cold and about 5 cc. of water had been added,

(21) Willard and Thompson, THIS JOURNAL, **52**, 1893 (1930).

it was opened and the contents were washed into a 100-cc. beaker. Exactly 25 cc. of 0.1169 *N* sodium hydroxide was added. After the mixture had stood for twenty minutes (to allow for the complete hydrolysis of the unused acetic anhydride), the excess alkali was titrated with 0.1016 *N* hydrochloric acid until the phenolphthalein pink had just been dissipated (a micro buret was used in this titration).

Blanks were run and test determinations were made using 3-phenyl-5-*p*-bromophenylisoxazoline, *syn*-styryl-*p*-bromophenylketoxime, and cholestenoneoximes "A" and "B" (Table II).

The application of the method outlined to our particular compounds was not wholly satisfactory because some decomposition occurred. When the results were corrected for the hydrogen bromide liberated (actually determined) during the whole process, it was evident that no acetic anhydride had combined with the brominated cholestenoneoximes or their derivatives (Table II).

The Preparation of the Acetate of Cholestanoneoxime "B."—Five-tenths gram of cholestenoneoxime was dissolved in 5 cc. of dioxane and 5 cc. of acetic anhydride was added. At the end of three hours at 80–85°, the solution was poured into iced water, whereupon an oil separated. The oil was taken up in ether which was then thoroughly washed and dried with anhydrous sodium sulfate. The dry solution was carefully evaporated *in vacuo*. An unsuccessful attempt was made to crystallize the residue from a mixture of methyl and ethyl alcohols. The alcohol was then removed by evacuation at 7 mm. at a temperature of 65°. After three hours, it was assumed that all the solvent had been removed. A glassy product resulted.

The above preparation was repeated except that the reaction was allowed to run for seventy-two hours at room temperature. This preparation yielded a crop of unctuous crystals when the ethyl–methyl alcohol solution was cooled to −16.5°. However, they changed to oil when an attempt was made to filter them off. For this reason, the solvent was removed in the manner described above. A glassy product was obtained here also.

The refractive index, n_D, of the two above products was measured at 60, 55, 50, and 45° (Table III).

The Preparation of the Acetate of Cholestenoneoxime "A."—This preparation was effected in the manner described above, except that glacial acetic acid was used as the solvent for the oxime. (Solution was effected by gently shaking a suspension of cholestenoneoxime in glacial acetic acid. If the shaking was continued too long, cholestenoneoxime "A" separated out and its resolution always required the application of heat.) As before, one preparation was made at 80–85° and the other at room temperature. Glassy products resulted from both preparations. The refractive indices were measured at the same temperatures as above (Table III).

The Determination of the Index of Refraction of Alcoholic Solutions of, and the Calculation of the Specific and Molecular Refractions, of Cholestenoneoximes "A" and "B."—1. The density and the index of refraction of an approximately 95% alcohol were determined at 25°. From these values, the specific refraction, $R^{25}D$, was calculated[22,23]

$d^{25}_4 = 0.8055 \pm 0.0003$ (four determinations)
$n^{25}D = 1.3619$ (ten determinations)

$$R^{25}D = \frac{n^2 - 1}{n^2 + 2} \times \frac{1}{d} = 0.27529$$

2. A 1.227% solution of cholestenoneoxime "A" in the above alcohol was prepared using 0.10045 g. of oxime and 8.08803 g. of alcohol. After measuring d and n, the R of the solute was calculated by means of the law of mixtures, in which $R_{(solution)} = R_{(solvent)} \frac{100 - p}{100} + R_{(solute)} \frac{p}{100}$, where p is per cent. (by weight) of solute.

$d^{25}_4 = 0.8083 \pm 0.0003$ (three determinations)
$n^{25}D = 1.3636$ (eleven determinations)
$R^{25}D_{(solution)} = 0.27549$, $R^{25}D_{(solute)} = 0.2919$
$M^{25}D = 116.6$

3. A solution of cholestenoneoxime "B" (m. p. 151.8°) was prepared from 0.10040 g. oxime and 8.07283 g. of alcohol, giving a 1.228% solution. The same determinations and calculations as above were made.

$d^{25}_4 = 0.8072 \pm 0.0003$ (five determinations).
$n^{25}D = 1.3633$ (nine determinations).
$R^{25}D_{(solution)} = 0.27566$, $R^{25}D_{(solute)} = 0.3057$
$M^{25}D = 122.1$

The theoretical molecular refraction of cholestenoneoxime, obtained by adding the atomic refractions and assuming that the oxime is more nearly aliphatic than aromatic in nature, is 123.5.

Summary

1. Two forms of cholestenoneoxime exist. The one, "A," which is relatively the more labile, melts at 65°, solidifies again at about 85°, and remelts at 151.8°. The other, "B," is the ordinary stable form which melts at 151.8°.

2. The ordinary form can be converted to the less stable form by treatment with glacial acetic, propionic, or *n*-butyric acid.

3. The less stable form reverts to the more stable oxime in benzene or carbon tetrachloride, or upon being heated to a temperature above its melting point.

4. Either form can exist, without apparent change, in formic acid.

5. The lower melting form has a lower specific refraction and a lower molecular refraction than does the higher melting form.

6. It is believed that the compound which melts at 65° is *syn*-3-hydroxylimino-cholesten-4,5, in which the hydroxyl is *syn* with respect to the $\Delta^{4,5}$ double bond.

7. It is believed that the oxime melting at 151.8° is *anti*-3-hydroxylimino-cholesten-4,5, in which the hydroxyl is *anti* with respect to the $\Delta^{4,5}$ double bond.

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(22) Lorentz, *Wied. Ann.*, **9**, 641 (1880).
(23) Lorenz, *ibid.*, **11**, 70 (1880).