chloride or zinc in acetic acid, hydroquinone, or phenylhydrazine. Except for the tautomerism that enables lapachol, like 2-hydroxy-1,4-naphthoquinone, to form two kinds of ethers, the oxidations of XII to XI and lapachol to lapachol peroxide are identical.

Experimental

Absorption Spectra.—Measurements were made in U. S. P. chloroform solution with a Beckman Spectrophotometer, Model DU, used by courtesy of Dr. I. H. Scheinberg. The positions and intensities of maxima were: lapachol peroxide, 250 mμ (log ε 4.48), 289 (4.37), 340 (3.73) (inflection), 400 (3.36) (inflection); lapachol peroxide monophenazine, 249 (4.61), 290 (4.39) (inflection), 332 (4.19), 410 (3.39) (inflection); lapazine, 280 (4.46) (inflection), 305 (4.61), 362 (3.69), 415 (4.00), 435 (3.97) (inflection).

Sensitivity to Alkali.—A solution of lapachol peroxide in chloroform or ether imparted no color to 0.1 *N* sodium hydroxide, but if alcohol or dioxane was added to the mixture, a deep red color appeared within a second. Under the same conditions, 2-methoxy-1,4-naphthoquinone required roughly a minute for hydrolysis, lapachol peroxide monophenazine two minutes, and lapachol methyl ether an hour.

A chloroform solution of lapachol peroxide, treated with an equal amount of 2% sodium bicarbonate and sufficient alcohol (two volumes) to homogenize the mixture, developed a strong red color in a minute. No instantaneous color appeared if a solution of the peroxide in aqueous alcohol–chloroform was treated with bicarbonate after standing five minutes.

Lapachol Peroxide Monophenazine.—A mixture of 0.5 g. of lapachol peroxide, 0.25 g. of *o*-phenylenediamine, and 10 cc. of acetic acid was heated on the steam-bath for half an hour, cooled, and filtered. The solid, crystallized from acetic acid (100 cc. per g.), afforded 0.45 g. (78%) of small, bright yellow needles of lapachol peroxide monophenazine, m. p. 184–185° (dec.). The analytical sample darkened at 182° and melted at 185–186° (dec.) (bath preheated to 175°).

Anal. Calcd. for $C_{28}H_{30}O_4N_2$: C, 77.96; H, 5.45; N, 5.05. Found: C, 78.18; H, 5.12; N, 5.31.

Lapachol peroxide monophenazine is sparingly soluble in cold acetone, alcohol, or ether, readily in chloroform and hot benzene or dioxane. In concentrated sulfuric acid it forms an orange-yellow solution that darkens rapidly to blackish green. If a solution of the phenazine in aqueous dioxane is treated with sodium bisulfite, the color immediately deepens toward orange, possibly because of cleavage to lapeurhodone.

After a mixture of 0.1 g. of lapachol peroxide, 0.1 g. of *o*-phenylenediamine and 10 cc. of acetic acid was refluxed for ten minutes, cooled and diluted with 3 cc. of water, the precipitated oil soon solidified and yielded 0.09 g. of recrystallized monophenazine. Further dilution of the mother liquor with water afforded 0.01 g. of crude lapeurhodone.

Hydrolysis of Monophenazine.—A mixture of 0.2 g. each of lapachol peroxide monophenazine and zinc dust was heated under reflux for fifteen minutes with a solution of 1 g. of potassium hydroxide pellets in 5 cc. of water and 20 cc. of alcohol, cooled and filtered, and the intensely crimson filtrate poured into excess dilute acid and extracted with ether. The ether solution was extracted with 5% sodium carbonate, washed with water, dried and evaporated, and the residue crystallized from 2 cc. of alcohol. There was obtained 92 mg., m. p. 158–162°, of dark red crystals contaminated with a yellow solid, possibly dehydrolapazine,[3] a known reaction product of alkali and lapeurhodone. Recrystallization from 5 cc. of alcohol afforded 63 mg. (56%) of dark red, hexagonal tablets of lapeurhodone, m. p. 165.5–167°, which gave a deep green solution in concentrated sulfuric acid and did not depress the melting point of an authentic sample.

The sodium carbonate extracts were washed judiciously with ether, acidified hot, chilled, and filtered. The precipitate, crystallized from 1 cc. of alcohol, furnished 75 mg. (86%) of golden plates of lapachol, m. p. 141–142.5°, which did not depress the melting point of the genuine substance.

Summary

The structure of lapachol peroxide, a derivative of 1,2,4-triketotetralin and an *o*-quinonoid ether of lapachol, is elucidated.

CAMBRIDGE 38, MASSACHUSETTS
RECEIVED JANUARY 11, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMICAL ENGINEERING, UNIVERSITY OF TORONTO, AND THE DOMINION RUBBER COMPANY, RESEARCH LABORATORIES]

Separation of the Acids of Hog Bile

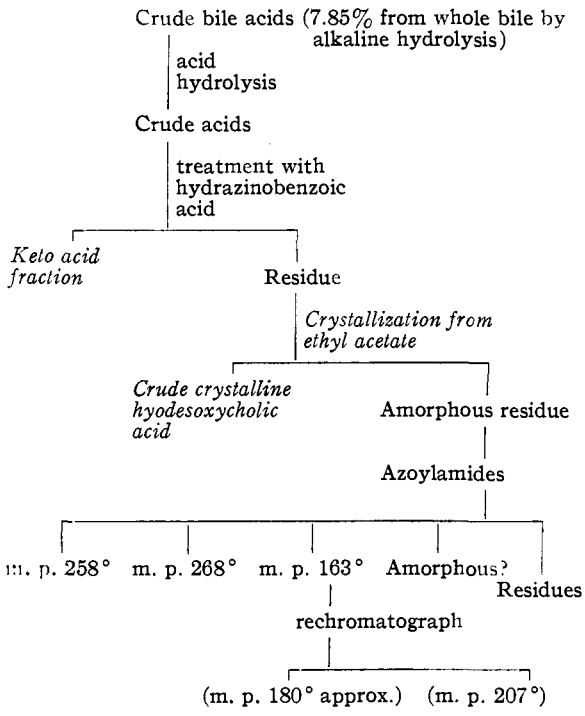
BY E. BRUCE TRICKEY[1]

Although the composition of the bile acid mixtures from other commonly available animals is well-known, that of the hog is a notable exception.

Numerous preliminary experiments led to the development of a chromatographic method for the separation of the hog bile acids. Whole hog bile was subjected to an alkaline hydrolysis to release the unconjugated acids which were recovered as a dark-brown resinous mixture in 7.85% yield based on the starting weight of the whole bile. This material was called the "crude bile acids" and served as the starting point in our attempts at separation. Percentage yields of products reported in this paper are calculated on the basis of the weight of "crude bile acids." This product was subjected to an acid hydrolysis, followed by a partial removal of the keto acids with hydrazinobenzoic acid. The dry residue deposited hyodesoxycholic acid in 22.4% yield from ethyl acetate. The amorphous fraction was converted to the acid chlorides of the bile acid formates and reacted with aminoazobenzene to obtain the azoyl amide formates. On deformylation and chromatographing over alumina four main products were obtained which were numbered in the order of their appearance in the filtrate. Band I was found to be derived from 3-hydroxy-6-keto-*allo*cholanic acid. Band II an-

(1) This paper comprises a portion of a thesis presented by E. Bruce Trickey in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemical Engineering, University of Toronto. Present address: Ardentown, Delaware.

DIAGRAM I



alyzed correctly for a dihydroxycholanic acid azoylamide, possibly chenodesoxycholic acid. Band III was found to be impure. On rechromatographing, the azoyl amide of hyodesoxycholic acid was obtained in pure condition along with a small amount of a closely related impurity. Band IV was of doubtful crystallinity. The analytical figures were not consistent with a cholanic acid nucleus. The empirical formula arrived at from the analysis was $C_{33}H_{53}O_5N_3$. From this the parent acid must have the formula $C_{27}H_{44}O_6$, which suggests a steroid sapogenin.

The weights of the fractions obtained in the chromatogram are shown in the table. The percentage of each component in the mixture of crude bile acids was calculated from the chromatogram and from the weights of products isolated before chromatographing.

Calculation of the Percentage Composition of the "Crude Hog Bile Acids."—On examination it was found that the intermediate fraction between the aminoazobenzene and Band I was a mixture of these two which could be separated by boiling with 60–90° petroleum ether. The aminoazobenzene and Band I were therefore transferred to their appropriate fractions. It was also found that a small amount of aminoazobenzene was in Band I, therefore it was combined with its corresponding fraction. When this was done the new weights were: aminoazobenzene fraction, 0.36 g., Band I, 2.20 g.

Chromatography had shown that there were four main components of the resinous part of the "crude bile acids." For the purpose of a first approximation to the percentage composition it was assumed that the weights of material collected in each intermediate fraction belonged to the preceding band (Table I). The percentage of each fraction in the starting weight of 9.64 g. of azoylamides is shown in column (4), Table I, which is also the percentage composition of the acids in the resin from 4. To obtain the percentage of each in the original mixture of bile acids the figures in column (4) were reduced by the factor 107.5/183, the weight of the resin and "crude bile acids," respectively. The results are shown in column (5). Since Band I was shown to be a keto acid, the total keto acids present were 17.7 plus 3.44 = 21.14%. Also Band III was shown to be derived from hyodesoxycholic acid and therefore the total hyodesoxycholic acid present was 22.40 plus 16.40 = 38.80%. The other constituents are as shown in the table.

TABLE I

Fraction	Wt. of fractions	Adjusted wt. for calcn.	% of resin from 4	% of crude bile acids
Forerun	0.35	0.35	3.6	2.13
Aminoazobenzene	0.25	0.36	...	...
Intermediate	0.16	...	...	...
Band I	2.15	2.90	30.1	17.7
Intermediate	0.70			
Band II	1.09	1.38	14.3	8.40
Intermediate	0.29			
Band III	1.8	2.69	27.9	16.40
Intermediate	0.89			
Band IV	.74	0.83	8.6	5.05
Intermediate	.09			
Residues	.69	0.69	7.2	4.21
Loss on alumina	.80	0.80	8.3	4.88
Total	10.0	10.00	100.0	58.77
Hyodesoxycholic acid (by crystallization				22.40
Keto acids (by *p*-carboxyphenylhydrazine)				3.44
Total accounted for				84.61
Mechanical losses				15.39

Experimental Part[2]

1. **Caustic Hydrolysis.**—Hog bile (4665 ml.) was evaporated on a steam-bath with 230 g. of flake sodium hydroxide to a volume of 1500 ml. Potassium hydroxide (200 g.) was added and the solution heated on the steam-bath for three hours. This solution was transferred to an autoclave and heated with one liter of ethyl alcohol at 140° for six hours with shaking. After cooling overnight the autoclave was opened and the clear, dark brown solution neutralized with strong hydrochloric acid to give a very slightly basic solution. The separated slime was filtered off on a bed of Filter-Cel in a large Buchner funnel. The clear brown filtrate was strongly acidified and the precipitated acids separated from the water layer by decantation. Excess mineral acid was removed by kneading the doughy lump in cold water. By stirring this product in two liters of hot water containing 50 g. of sodium acetate, a smooth mixture was obtained from which the sterols and fatty acids were removed by vigorous stirring with one

(2) All melting points were taken with an Anschütz type thermometer without stem correction.

liter of benzene. The crude acids were kneaded once more in cold water and then completely dried at 150°. The dry product (366 g., 7.85%) was a brownish, brittle resin.

2. **Acid Hydrolysis.**—Our procedure was patterned after that of Power[3] for sterol glycosides.

The dry, ground, hog bile acids, weighing 183 g., were refluxed three hours with 1500 ml. of *n*-amyl alcohol, 300 ml. of ethanol and 500 ml. of 18% hydrochloric acid. At the end of three hours one liter of water was added and the alcohol steam distilled, leaving a sticky, resinous, dark mass and a reddish water layer. An excess of flake sodium hydroxide was added and dissolved by heating. Enough ethyl alcohol was added to give a homogeneous solution, which was heated for one hour. Steam was passed through the hot solution to remove the alcohols. The solution was cooled to 20° and acidified with dilute hydrochloric acid. A finely divided, non-gummy mixture of acids separated and was filtered, washed and dried at 150° to a brown resin weighing 176 g.

A sample of this product dissolved in ethyl acetate deposited crystalline material sintering at 187° and melting at 193°. However, since it had been found that removal of the keto acids helped crystallization markedly, these were partially removed before crystallization of the hyodesoxycholic acid.

3. **Partial Removal of the Keto Acids and Hyodesoxycholic Acid.**—One hundred seventy-three grams of the crude acids were treated according to the procedure Anchel and Schoenheimer[4]; 9.0 g. of hydrazones was obtained corresponding to a yield of keto acids of 6.3 g. or 6.3/183 × 100% = 3.44%. The residue from this treatment was hydrolyzed with potassium hydroxide in alcohol to regenerate the acids which then weighed 150 g. On grinding this product to a fine powder and boiling with 200 cc. of ethyl acetate, 41 g. (41/183 × 100% = 22.4% yield) of hyodesoxycholic acid was obtained which on one recrystallization melted at 197°. The non-crystalline residue weighed 107.5 g.

4. **Preparation of the Azoylamides.**—Twenty-five grams of the resinous acids from 3 was converted to the acid chlorides of the bile acid formates according to the procedure of Hoehn and Moffett.[5]

The residue was taken up in 150 cc. of dry benzene and to this solution was added 24.1 g. of recrystallized aminoazobenzene (m. p. 125–126°) in 550 cc. of dry benzene and 40 cc. of ether. There was an immediate dark red precipitate of aminoazobenzene hydrochloride. The mixture after standing overnight was filtered through 13 g. of Filter-Cel and the filtrate evaporated to dryness.

The weight of orange resin obtained was 41.5 g. This was dissolved in 2 liters of hot dioxane and treated with 40 g. of potassium bicarbonate in 300 cc. of water. After one hour and fifteen minutes the solution was diluted with cold water. The precipitated lump was removed and broken up by stirring with cold water for some time, filtered, dried and weighed (37.5 g., 94%).

5. **Chromatography of the Product from 4.**—An adsorption column was prepared by slurrying 1080 g. of activated alumina[6] with pure chloroform in a glass tube 4.5 by 80 cm., fitted with a stopcock on the bottom. The column of alumina after settling overnight was 4.5 by 70 cm. A 5-liter separatory funnel was joined tightly to the upper end of the column by means of a large rubber stopper.

A solution of 10 g. of the amides prepared as in 4, in 160 ml. of pure chloroform was added to the column. Development was begun with pure chloroform in the usual manner at a rate of 5 cc./min. It was found advantageous to hasten the development by addition of small quantities of methanol to the chloroform during the course of the development. Fractions were recovered in the filtrate by evaporation of the solvent. The fractions obtained are summarized in Table I.

Band I was recrystallized twice from chloroform and then melted at 257–258°.

Anal.[7] Calcd. for $C_{36}H_{47}O_3N_3$: C, 75.7; H, 8.27; N, 7.38. Found: C, 75.61, 75.51; H, 8.46, 8.09; N, 7.50.

A mixed melting point with the amide prepared by partial oxidation of hyodesoxycholic acid azoyl amide with chromic anhydride in acetic acid, showed no depression.[8]

Band II was recrystallized from a methanol–chloroform mixture to give yellow prisms melting sharply at 267–268°.

Anal. Calcd. for $C_{36}H_{49}O_3N_3$: C, 75.7; H, 8.58; N, 7.35. Found: C, 75.61, 75.75; H, 8.39, 8.39; N, 7.56, 7.56.

Band III was twice recrystallized from chloroform but the melting point range was still quite broad with considerable softening as low as 165° with some still not melted at 190°.

Anal. Calcd. for $C_{36}H_{49}O_4N_3$: C, 73.6; H, 8.35; N, 7.16. Found: C, 73.75, 73.89; H, 8.00, 8.11; N, 7.08, 7.06.

The analysis seemed to indicate a trihydroxyazoylamide but the melting point behavior was so characteristic of the amide of hyodesoxycholic acid that this product was rechromatographed on a column of alumina 4.5 × 60 cm. using 2% methanol in chloroform. The lower component of the main fraction comprised about one-quarter of the total and had a low and broad melting point. It was not further purified. The upper component was collected in two parts, the first having a melting point of 206° with slight previous sintering, the second melting sharply at 206–207°. A mixed melting point with hyodesoxycholic acid azoylamide was not depressed.

Band IV was very soluble in methanol or chloroform but nearly insoluble in benzene. A solution of Band IV in benzene containing a little chloroform was filtered from a trace of dark, insoluble material. On cooling the solution, a strong gel formed which was not broken by addition of small amounts of methanol. Therefore, this product was twice precipitated from a warm benzene–chloroform solution with 60–90° petroleum ether and then melted in the range 150–160°.

Anal. Calcd. for $C_{39}H_{53}O_5N_3$: C, 72.78; H, 8.28; N, 6.53. Found: C, 72.75, 72.51; H, 8.40, 8.24; N, 6.60, 6.55.

Acknowledgment.—The author wishes to express his sincere gratitude and appreciation to Dr. R. H. F. Manske and Professor J. G. Breckenridge for their kindly assistance and encouragement in the course of this work. Grateful acknowledgment is also made to the School of Engineering Research for financial assistance during 1945.

Summary

The yield of crude bile acids from the hog was found to be 7.85%.

By a combination of crystallization and chromatography of the azoylamides of the non-crystalline fraction the crude acids of hog bile have been separated and shown to consist of approximately 40% hyodesoxycholic acid, 20% of keto

(3) Power and Solway, *J. Chem. Soc.*, **103**, 399 (1913).

(4) Anchel and Schoenheimer, *J. Biol. Chem.*, **124**, 609 (1938).

(5) Hoehn and Moffett, THIS JOURNAL, **67**, 740 (1945).

(6) The alumina used was supplied by the Harshaw Chemical Co., 1945 East 97th St., East Cleveland 6, Ohio. The grade used was Al-1 powder activated alumina.

(7) The author is indebted to Mr. A. Ledingham of the Dominion Rubber Research Labs. for the microanalyses.

(8) In another experiment, the azoyl amide formates were chromatographed over alumina. The ester groups were hydrolyzed by the mildly basic alumina. In this case a second monohydroxy, monoketo azoylamide was obtained which melted at 226°. Boiling this material with potassium bicarbonate in dioxane–water solution converted it to the azoyl amide of 3(α)-hydroxy-6-keto-*allo*-cholanic acid, melting at 258°. Therefore, the 226° keto acid must be derived from 3(α)-hydroxy-6-ketocholanic acid.

acids mostly 3(α)-hydroxy-6-keto-*allo*cholanic acid, 9% of an unknown dihydroxycholanic acid, probably chenodesoxycholic acid and about 6% of a C_{27} acid, possibly a steroid sapogenin, as well as some residues not further examined.

GUELPH, ONTARIO RECEIVED JULY 27, 1948

[CONTRIBUTION FROM THE WHITMORE LABORATORIES, SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Derivatives of Benzofuran

BY R. B. WAGNER AND JOHN M. TOME

During studies of preparative procedures for benzofuran derivatives and for their use as precursors for the biosynthesis of penicillin,[1] 2-benzofurylacetic acid[2] was prepared by the Arndt–Eistert synthesis[3] in 29% yield from α-benzofuroic acid. Catalytic rearrangement of 2-diazoacetylbenzofuran (I) prepared from the latter gave ethyl 2-benzofurylacetate in 64% yield while rearrangement to the corresponding amide gave only a 34% yield. The 2-benzofurylacetic acid was obtained by hydrolysis of the ester.

A study of the reactivity[4] of the diazoketone (I) showed that treatment with aqueous hydrochloric acid yields 2-chloroacetylbenzofuran (II), hydrolysis with aqueous sulfuric acid gives 2-hydroxyacetylbenzofuran, and reduction with hydriodic acid yields 2-acetylbenzofuran. Treatment of the diazoketone (I) with glacial acetic acid or treatment of the chloroketone (II) with sodium acetate gave 2-hydroxyacetylbenzofuran acetate.

The chloroketone (II) was characterized by degradation to α-benzofuroic acid by treatment with pyridine to form α-benzofuroylmethylpyridinium chloride followed by alkaline cleavage. This reaction has been shown to be quite general.[5,6,7] Treatment of the chloroketone (II) under conditions of the haloform degradation reaction gave the same acid. The chloroketone was coupled with mercaptoacetic acid in the presence of base to give α-benzofuroylmethylmercaptoacetic acid.

Acknowledgment.—The authors are grateful to Professor H. D. Zook for his interest and valuable suggestions.

Experimental[8]

2-Diazoacetylbenzofuran (I).—An ethereal solution of 0.92 mole of diazomethane[9] was treated by the standard procedure[3] with an ether solution of 55.5 g. (0.31 mole) of α-benzofuroic acid chloride, m. p. 52–53°, b. p. 112–123° (6 mm.), 98–100° (3 mm.) (prepared as previously described).[2,10] A yield of 42.1 g. (74%) of yellow crystals, m. p. 111–118°, was obtained by crystallization from benzene–pentane. Recrystallizations from benzene–pentane and acetone gave a constant melting point of 117–118°.

Anal. Calcd. for $C_{12}H_8O_2N_2$: N, 15.05. Found: N (Dumas), 15.00, 15.11.

Ethyl 2-Benzofurylacetate by Rearrangement of the Diazoketone (I).—To a warm ethanolic solution of 20 g. (0.107 mole) of 2-diazoacetylbenzofuran was added 9.6 g. of silver oxide by the standard procedure.[3] The volume of gas collected during the reaction was 4% below the theoretical amount. The product was fractionated to give 14.1 g. (64% yield) of colorless liquid, b. p. 147–148° (8 mm.), m. p. 18.0–18.5°, $n^{20}D$ 1.5400, d^{20} 1.140.

Anal. Calcd. for $C_{12}H_{12}O_3$: C, 70.57; H, 5.93; M_D, 56.23. Found: C, 70.75; H, 6.36; M_D, 56.22. The calculated molecular refraction includes an exaltation correction[11] of +1.58.

The free acid, 2-benzofurylacetic acid, was obtained by refluxing for seventeen hours a solution of 8.2 g. of the ester and 14 g. of potassium hydroxide in 200 ml. of methanol. The mixture was diluted with an equal volume of water, concentrated in vacuum, and filtered. The solution was acidified to approximately *p*H 7 and the red gum which separated was discarded. The solution was then acidified to *p*H 3–5 (congo red paper) to give an orange precipitate. This precipitate was treated with activated carbon in ethanol and recrystallized first from ethanol, then from benzene–pentane to give 5.27 g. (75% yield) of white plates, m. p. 97–99° (lit.,[2] m. p. 98–99° cor.).

Anal. Calcd. for $C_{10}H_8O_3$: neut. equiv., 176. Found: neut. equiv., 179.

The amide was prepared from the 2-benzofurylacetic acid by a standard procedure,[12] giving white crystals from benzene, m. p. 163–164°. A mixed melting point determination with the amide from the rearrangement of the diazoketone (I) showed no depression.

2-Benzofurylacetamide by Rearrangement of the Diazoketone (I).—A hot solution of 5.0 g. of 2-diazoacetylbenzofuran, 50 ml. of dioxane and 50 ml. of concentrated ammonium hydroxide (sp. gr. 0.9) was treated with 27 ml. of 10% aqueous silver nitrate according to the standard procedure.[3] The volume of nitrogen collected was 6% below the theoretical amount. The product after treatment with activated carbon was recrystallized from benzene and from chloroform to yield 1.6 g. (34%) of white plates, m. p. 163.5–164.5°.

Anal. Calcd. for $C_{10}H_9O_2N$: N, 8.00. Found: N (Dumas), 8.07, 8.35.

2-Chloroacetylbenzofuran (II) **by Hydrohalogenation of** I.—To a solution of 2.0 g. of 2-diazoacetylbenzofuran in 7 ml. of dioxane was added 5 ml. of concentrated hydro-

(1) Stone, Patterson and Farrell, *J. Bact.*, **51**, 598 (1946).

(2) Reichstein and Reichstein, *Helv. Chim. Acta*, **13**, 1275 (1930).

(3) Bachmann and Struve, "Organic Reactions," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1942, p. 38.

(4) Eistert, "Newer Methods of Preparative Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1948, p. 513.

(5) King, THIS JOURNAL, **66**, 894 (1944).

(6) Reich and Reichstein, *Helv. Chim. Acta*, **22**, 1124 (1939).

(7) Brown, *Iowa State Coll. J. Sci.*, **11**, 221 (1936).

(8) All melting and boiling points are uncorrected.

(9) Arndt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 165.

(10) Fuson, Kneisley and Kaiser, "Organic Syntheses," Vol. 24, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 33.

(11) Auwers and Kohlhaas, *J. prakt. Chem.*, **108**, 321 (1924).

(12) Shriner and Fuson, "The Systematic Identification of Organic Compounds," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1940.