weak local anesthetic action, are moderately irritating, and much more toxic than procaine (LD-50 of Procaine HCl = 57 mg./kg. i. v. in mice). The toxicity is equal that of Butyn (LD-50 = 12 mg./kg.).

These results are interesting in view of the previous report by Gilman and Pickens[6] that VI was one-sixth as active as procaine. It is also curious that γ-di-*n*-butylaminopropyl 3-thenoate (IV) is slightly less active than the corresponding 2-thenoate.

## Experimental

**Acid Chlorides.**—The 3-thenoyl chloride was prepared from 3-methylthiophene by bromination with N-bromosuccinimide, conversion to the aldehyde by the Sommelet reaction, oxidation of the aldehyde with silver oxide, and conversion to the acid chloride with thionyl chloride, as previously described.[3] 2-Thenoic acid,[11] prepared by hypochlorite oxidation of 2-acetothienone, was also converted to the acid chloride satisfactorily with thionyl chloride.

(11) We are indebted to H. Grose, of this Laboratory, for a supply of 2-thenoic acid.

**Ester Hydrochlorides.**—The acid chlorides were allowed to react with the appropriate dialkylaminoalkanol in refluxing benzene, and after cooling the ester hydrochlorides were precipitated with dry ether. Samples of each of the six ester hydrochlorides were converted to the free bases by treating their water solutions with sodium carbonate and extracting the bases with ether. The physical constants of the salts and free bases are reported in Table I.

## Summary

The β-dimethylaminoethyl, β-diethylaminoethyl, β-di-*n*-butylaminoethyl, γ-di-*n*-butylaminopropyl and β-morpholinoethyl esters of 3-thenoic acid have been prepared and characterized. The γ-di-*n*-butylaminopropyl ester of 2-thenoic acid was also prepared and characterized.

Of these, only γ-di-*n*-butylaminopropyl 2-thenoate and γ-di-*n*-butylaminopropyl 3-thenoate showed any activity as topical anesthetics on the rabbit cornea. The toxicities of the thiophene analogs are in the same order as the *p*-aminobenzoates.

BLOOMINGTON, INDIANA RECEIVED APRIL 30, 1948

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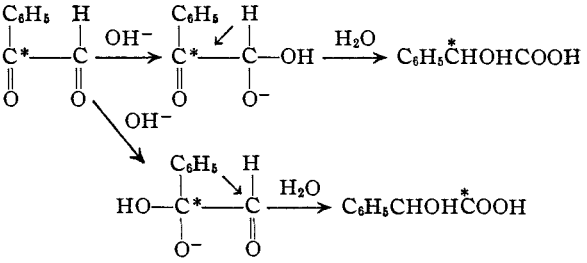
[CONTRIBUTION FROM OAK RIDGE NATIONAL LABORATORY AND THE UNIVERSITY OF TENNESSEE]

# C¹⁴ Tracer Studies in the Rearrangements of Unsymmetrical α-Diketones: Phenylglyoxal to Mandelic Acid[1]

BY O. KENTON NEVILLE

The reaction of phenylglyoxal with aqueous alkali to form mandelic acid has been shown to follow the same second-order kinetics[2] as the benzilic acid rearrangement. The latter reaction is known from the work of Westheimer[3] and of Roberts and Urey[4] to involve the reversible addition of hydroxyl ion at one of the carbonyl groups, followed by a group migration. A similar mechanism in the case of phenylglyoxal allows two possible modes of reaction: one in which the aldehydic hydrogen shifts following hydroxyl ion addition at the aldehyde group, and a second in which a phenyl group migrates as the result of hydroxyl ion addition at the keto group. The former appears to be more probable since Gray and Fuson[5] have found that mesitylglyoxal, in which the keto group is shielded from hydroxyl ion attack, reacts smoothly to give mesitylglycolic acid.

A decision between the two possible mechanisms is possible by the device of labelling one of the carbonyl groups with isotopic carbon, since the two modes of reaction will lead to isotopically distinguishable products.




Accordingly, phenylglyoxal, labelled in the keto carbonyl group with C¹⁴ has been studied in its reaction with aqueous alkali, and is shown to be converted to mandelic acid without rearrangement of the carbon skeleton.[6]

Very recently, Doering, Taylor and Schoenewaldt[7] have reported that mandelic acid containing no deuterium was obtained from the reaction of phenylglyoxal in basic deuterium water, thus showing that, if hydrogen migrates, it must do so intramolecularly. In addition, they have shown, with the aid of C¹³, that when carbonyl-labelled α,α-dibromoacetophenone is allowed to react with aqueous base, similarly labelled mandelic acid is obtained. Previous to their publication, we had obtained, with the aid of C¹⁴-labelled α,α-dibromoacetophenone, results which are in agree-

(1) This document is based on work performed under Contract No. W-7405 eng 26 for the Atomic Energy Project at Oak Ridge National Laboratory. A part of the work herein described has been submitted, under the graduate study program of the Oak Ridge Institute of Nuclear Studies, to the University of Tennessee in partial fulfillment of the requirements for the Ph.D. degree.

(2) Alexander, THIS JOURNAL, **69**, 289 (1947).

(3) Westheimer, *ibid.*, **58**, 2209 (1936).

(4) Roberts and Urey, *ibid.*, 60, 880 (1938).

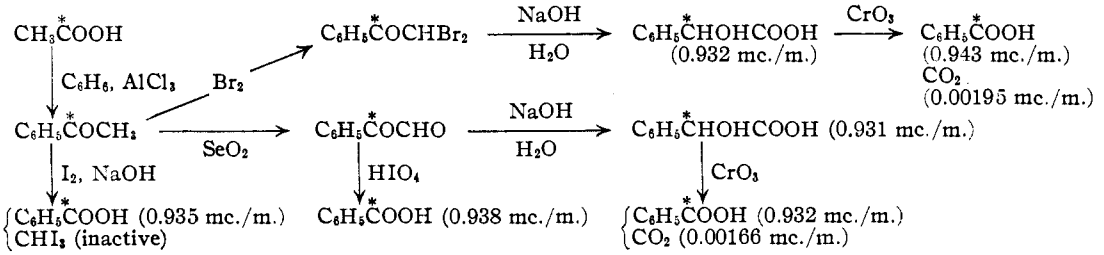
(5) Gray and Fuson, *ibid.*, **56**, 739 (1934).

(6) The results of this work were reported before the Division of Organic Chemistry of the American Chemical Society at the New York Meeting, September 19, 1947.

(7) Doering, Taylor and Schoenewaldt, THIS JOURNAL, **70**, 455 (1948).

ment with theirs. Since the techniques used by us differ substantially from those reported, we feel it would be of value to report this work together with the results of the investigation of the phenylglyoxal reaction.

The syntheses and degradations used in studying the two reactions were carried out as shown in the scheme below. The radioactive assays, expressed in terms of millicuries per mole (mc./m.), were based on ionization chamber measurements of the carbon dioxide formed by the wet oxidation of 3–8 mg. samples. The factor for the conversion of ion current, as determined on the dynamic condenser electrometer,[8] is subject to some uncertainty, but we have, nevertheless, transposed the measured results in order to indicate the level of activity in terms of millicuries per mole (mc./m.).




Each step in the scheme was carefully worked out with inactive materials before the experiment with radioactive compounds was performed. The carbonyl-labelled acetophenone was prepared from carboxyl-labelled acetic acid[9] by a modification of the method of Groggins, Nagel and Stirton.[10] Phenylglyoxal, prepared by selenium dioxide oxidation[11] of acetophenone, was not isolated due to large losses on the small scale, but was converted directly to mandelic acid by shaking the ether solution with aqueous sodium hydroxide.

The carbon dioxide formed by chromic acid oxidation of mandelic acid at room temperature was precipitated as barium carbonate, which was dried and weighed before it was decomposed to carbon dioxide for radioactive assay. Less than 1% of the radioactivity in the mandelic acid was present in the carbon dioxide. It is interesting to note that when the oxidation was carried out with alkaline permanganate, 8% of the original activity was found in the carbon dioxide. The quantity of the carbon dioxide in this experiment was not ascertained, but since the molar activity of the benzoic acid fraction was equal to that of the starting material, it is evident that the active carbon dioxide must have been formed either by the further degradation of benzoic acid or by attack by permanganate on mandelic acid at points other than the α-carbon–carboxyl bond.

The α,α-dibromoacetophenone was prepared by bromination of a carbon disulfide solution of carbonyl-labelled acetophenone in the presence of light. It was converted to mandelic acid by overnight contact with 5% sodium hydroxide solution. The chromic acid oxidation of mandelic acid to benzoic acid was carried out in a similar manner to that of the mandelic acid from phenylglyoxal.

**Acknowledgments.**—The author is indebted to Dr. W. G. Brown and Dr. G. E. Boyd for their assistance and encouragement with the problem, and to Mr. C. J. Borkowski and Mr. J. K. East for their assistance and advice in the use of the electrometer.

## Experimental

**Carbonyl-labelled Acetophenone.**—Three grams of carboxyl-labelled acetic acid was boiled under reflux with 30 g. of benzene and 20 g. of anhydrous aluminum chloride for eight hours. The mixture was decomposed in the reaction flask with a water-hydrochloric acid solution. After allowing the two layers to stand in contact overnight, the benzene layer was separated and washed with 10% sodium hydroxide solution. The water and benzene were removed by distillation through a short column, and the high boiling fraction, which distilled at 195–199°, was collected; the yield was 4.73 g. or 78.8% of the theoretical.

**Phenylglyoxal.**—One gram of carbonyl-labelled acetophenone, 1.66 g. of selenium dioxide, 6 ml. of dioxane and 0.2 ml. of water were boiled under reflux for eight hours. The solution was transferred from the precipitated selenium with a capillary pipet, and the solvent was distilled through a short column leaving the solid phenylglyoxal hydrate.

**Mandelic Acid from Phenylglyoxal.**—The crude phenylglyoxal was not purified, but was dissolved in ether and shaken with 30 ml. of 10% sodium hydroxide solution for one hour. The solution was neutralized and the product was precipitated as cadmium mandelate. The mandelic acid, recovered by solution of the salt in concentrated hydrochloric acid and ether extraction, was recrystallized from benzene; yield 0.9488 g. or 75.2%, m. p. 117–118°. Radioactive assay: 4.745 mg. produced $1.075 \times 10^{-13}$ ampere ionization current.

**Chromic Acid Oxidation of Mandelic Acid.**—A 0.0453 g. sample of mandelic acid, dissolved in 5 ml. of water and 0.4 g. of sulfuric acid, was allowed to react at room temperature for one hour with 0.2 g. of chromic anhydride. The reaction vessel was connected through a short water-cooled condenser and a Dry Ice trap to two bubbler tubes filled with half-saturated barium hydroxide solution. During the thirty-minute oxidation period, nitrogen was allowed to bubble slowly through the reaction mixture at room temperature in order to sweep the evolved carbon dioxide into the barium hydroxide solution. At the end of this time, the precipitated barium carbonate was centrifuged, washed and dried. The weight was 0.0364 g. or 62% of theoretical. Assay of the carbon dioxide produced

(8) For a description of this instrument see Palevsky, Swank and Grenchik, *Rev. Sci. Instruments*, **18**, 298 (1947).

(9) The labelled acetic acid was prepared by L. B. Spector (present address: Department of Chemistry, Harvard University) by a Grignard synthesis using methyl iodide and radioactive carbon dioxide.

(10) Groggins, Nagel and Stirton, *Ind. Eng. Chem.*, **26**, 1313, 1317 (1934).

(11) Riley and Gray, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., p. 509.

by acidification of the sample was $1.133 \times 10^{-15}$ ampere ionization current.

The reaction mixture was heated to boiling for five minutes (after the carbon dioxide absorbers were removed) in order to oxidize any remaining benzaldehyde to benzoic acid. The benzoic acid was removed by filtration: yield, 0.0280 g. or 77.7% of theoretical, m. p. 120–121°. Radioactivity assay: 6.71 mg. produced $1.895 \times 10^{-13}$ ampere ionization current.

**Permanganate Oxidation of Mandelic Acid.**—A 0.08832 g. sample of mandelic acid was boiled under reflux for one hour with 0.158 g. of potassium permanganate and 0.1234 g. of potassium hydroxide in 10 ml. of water. The carbon dioxide released by acidification of the solution was swept into the ionization chamber with inactive carbon dioxide. Radioactive assay: $1.6285 \times 10^{-13}$ ampere ionization current. The benzoic acid was recovered by ether extraction and recrystallization from water; yield, 0.60 g. or 85.2%, m. p. 120–121°. Radioactive assay: 4.49 mg. produced $1.266 \times 10^{-13}$ ampere ionization current.

**Benzoic Acid and Iodoform from Acetophenone.**—To 0.1 g. of acetophenone were added 1.2 g. of iodine, 0.82 g. of potassium iodide and 2 ml. of methanol in 5 ml. of water. One gram of sodium hydroxide was added in small portions to the constantly stirred solution. After the solution had stood for one hour the iodoform was filtered, washed with water and dried; yield, 0.305 g. or 92.8%, m. p. 118–119°. Radioactive assay: 19.29 mg. produced ionization current indistinguishable from that of background. The filtrate, upon acidification and treatment with sodium bisulfite, yielded 0.059 g. of benzoic acid or 58.4% of theoretical, m. p. 120–121°. Radioactive assay: 3.4 mg. produced $9.64 \times 10^{-14}$ ampere ionization current.

**Benzoic Acid from Phenylglyoxal.**—A 0.1-g. sample of acetophenone was boiled under reflux for four hours with 0.1 g. of selenium dioxide in 1.5 ml. of dioxane and was allowed to stand overnight. After transfer of the solution from the precipitated selenium and distillation of the solvent, the solid phenylglyoxal hydrate was allowed to react with 0.2 g. of periodic acid in one ml. of water. After standing one hour, the solution was filtered and the precipitated benzoic acid was recrystallized from water; yield, 0.065 g. or 64.4%; m. p. 120–121°. Radioactive assay: 4.38 mg. produced $1.245 \times 10^{-13}$ ampere ionization current.

**α,α-Dibromoacetophenone.**—To 1.2 g. of carbonyl-labelled acetophenone dissolved in 1.5 ml. of carbon disulfide was added slowly 3.2 g. of bromine under strong illumination from a photoflood lamp. After the absorption of bromine was complete, the solution was frozen in a Dry Ice–acetone-bath until crystallization occurred. The product was recrystallized once from a carbon disulfide–petroleum ether. The yield of crude α,α-dibromoacetophenone was 2.55 g. or 91.8% of theoretical.

**Mandelic Acid from α,α-Dibromoacetophenone.**—To this product was added 15 ml. of 5% sodium hydroxide, and the reaction mixture was allowed to stand at room temperature with stirring overnight. The slightly brown solution was extracted twice with chloroform and neutralized with hydrochloric acid. The mandelic acid was precipitated as cadmium mandelate, which was washed with ether and dried. The yield of cadmium mandelate was 1.81 g. or 87.5% of theoretical. The cadmium salt was dissolved in 20 ml. of concd. hydrochloric acid, diluted with a few drops of water and saturated with sodium chloride. The mandelic acid was extracted with four 10-ml. volumes of ether. After the ether was evaporated, the mandelic acid was recrystallized from a benzene–ether solution and dried in an Abderhalden drying pistol at 64° under vacuum. The yield was 1.3 g. or 85.5% of theoretical; m. p. 117°. Radioactive assay: 5.23 mg. produced $1.186 \times 10^{-13}$ ampere ionization current.

**Oxidation of Mandelic Acid from α,α-Dibromoacetophenone.**—A 0.06999 g. sample of this mandelic acid was oxidized in a similar manner to that described above for the mandelic acid from phenylglyoxal. The barium carbonate recovered was 0.0774 g. or 85.3% of the theoretical. The radioactive assay of the carbon dioxide from the barium carbonate was $2.85 \times 10^{-15}$ ampere ionization current. The benzoic acid recovered by ether extraction was recrystallized from water. The yield was 0.025 g. or 69.4% of theoretical, m. p. 121°. Radioactive assay: 4.86 mg. produced $1.391 \times 10^{-13}$ ampere ionization current.

**Microcombustions.**—Organic samples for radioactivity determinations were converted to carbon dioxide by a modification of the wet-oxidation method developed by Van Slyke, Folch and Plazin.[12] A diagram of our apparatus is presented in Fig. 1. The ionization chamber was placed in position I for evacuation and flushing with inactive carbon dioxide prior to a determination of the background radioactivity. The chamber was then evacuated and moved to position II. The sample (3–8 mg.) mixed with 200–400 mg. of potassium iodate was heated with 10–15 ml. of the Van Slyke combustion solution until all of the sample had disappeared (five to ten minutes), the released carbon dioxide passing through the pressure regulator into the evacuated chamber. Inactive carbon dioxide, which was allowed to bubble slowly through the solution during the combustion was then allowed to sweep through at a faster rate until atmospheric pressure was attained. This required three to five minutes. Figure 2 shows in detail

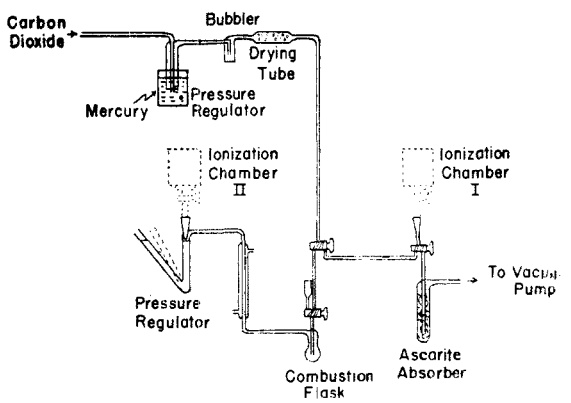



Fig. 1.—Combustion and chamber-filling apparatus.

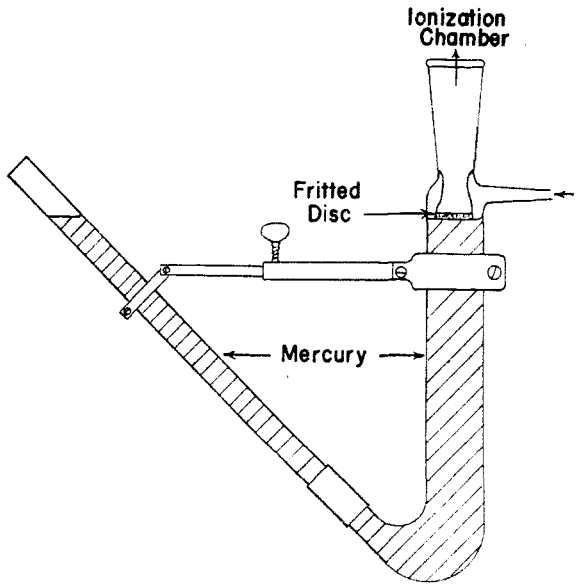



Fig. 2.—Pressure regulator.

(12) Van Slyke, Folch and Plazin, *J. Biol. Chem.*, **136**, 509 (1940)

the design of the pressure regulator[13] which permitted the gas to enter the evacuated chamber while the combustion was carried out at atmospheric pressure. The mercury valve allowed the passage of gas through the fritted disc when the pressure in the combustion flask rose above a value determined by the setting of the side-arm.

**Radioactivity Determinations.**—A dynamic condenser electrometer was used to determine the ionization current, the measured quantity being the voltage drop across a known high-valued resistor ($10^{11}$ ohms). To convert current readings to disintegrations per second, the factor $1 \times 10^{-16}$ ampere per disintegration per second was used, and these values were then converted to millicuries by the factor $3.7 \times 10^{7}$ disintegrations per second per millicurie.

The background current was determined prior to each filling of the chamber. It was found to remain practically constant at $5 \times 10^{-16}$ ampere, *i. e.*, negligibly small in comparison with the activities being determined.

In our procedure, a complete assay required about one hour, of which about fifteen minutes were taken in determining the background current, thirty minutes in weighing the sample and carrying out the combustion, and ten minutes in measuring the activity. The error in the analyses is estimated to be about 1%.

(13) This device was designed by Dr. W. B. Leslie, present address: Clovis, New Mexico.

## Summary

Mandelic acid, labelled $C^{14}$ in the $\alpha$-position, was obtained from similarly labelled phenylglyoxal on treatment with alkali, thus demonstrating that no rearrangement of the carbon skeleton occurs and consequently that the reaction proceeds by a shift of the aldehydic hydrogen atom. The similar reaction of $\alpha,\alpha$-dibromoacetophenone with aqueous alkali has also been shown to occur without rearrangement.

RECEIVED JUNE 1, 1948

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[CONTRIBUTION FROM THE LABORATORIES "SYNTEX," S. A.]

# Steroidal Sapogenins. I. Transformation of Kryptogenin into Diosgenin and Pseudodiosgenin

BY ST. KAUFMANN AND G. ROSENKRANZ

In the course of the past two years we have carried out in our Laboratories intensive studies on various steroidal sapogenins which appeared to us to be potential raw materials suitable for the manufacture of steroidal hormones. At that time, of all the known steroidal sapogenins, only diosgenin and its steroisomer, yamogenin, were of importance in the industrial preparation of hormones. In 1943, Marker, *et al.*,[1] described a new sapogenin, kryptogenin (I), isolated from the sapogenin fraction of extracts of Beth root. As this sapogenin occurs in important quantities in several species of Mexican dioscoreae as a constant companion of diosgenin and yamogenin, it was obvious to us that this natural product represented an important potential raw material and our efforts were concentrated on the transformation of this substance into other sapogenins or their derivatives suitable for the production of hormones.

Recently, Marker, *et al.*,[2] reported the reduction of kryptogenin by sodium and isopropyl alcohol and also by aluminum isopropylate and the subsequent isolation of diosgenin from the reduction mixture. On reproducing these reactions we found that only very small amounts of diosgenin are formed. In the course of the reduction of kryptogenin with sodium and alcohol the main reaction, as was to be expected, consisted in self-condensation of kryptogenin to fesogenin and subsequent reduction of this condensation product. In fact, we obtained fesogenin in good yield by refluxing kryptogenin with sodium alcoholate in alcoholic solution, in agreement with the observation of Marker, *et al.*,[3] who obtained fesogenin by the reaction of kryptogenin diacetate with alcoholic potassium hydroxide.

In order to establish the exact reduction conditions and to improve the yields of diosgenin, we submitted kryptogenin and its diacetate to catalytical hydrogenation using Raney nickel as catalyst in neutral medium. Marker, *et al.*, already have described the catalytical reduction of kryptogenin in acidic medium and in presence of platinum oxide as catalyst. By varying the conditions they obtained as main product 5,6-dihydrokryptogenin, tigogenin or dihydrotigogenin,[4] respectively.

We found that in neutral medium and in presence of Raney nickel only the ketonic group in 16 is reduced and we were able to isolate the 16-dihydrokryptogenin in good yield. This compound is comparatively unstable and very reactive. It is dehydrated by mineral or strong organic acids to diosgenin (III) and by boiling acetic anhydride to diosgenin acetate. The diacetate of 16-dihydrokryptogenin obtained by the catalytical hydrogenation of kryptogenin diacetate in the presence of Raney nickel in neutral medium is a colorless oil and can be saponified in alkaline solution to 16-dihydrokryptogenin. On the other hand, 16-dihydrokryptogenin diacetate is readily converted to pseudodiosgenin diacetate (IV) by dehydrating agents, such as phosphorus oxychloride, thionyl chloride, etc. The 16-dihydrokryptogenin can theoretically exist in three tautomeric forms (IIa, IIb, IIc). The above reactions can be illustrated by the formulas

(1) Marker, Wagner, Goldsmith, Ulshafer and Ruof, THIS JOURNAL, **65**, 739 (1943).

(2) Marker, Wagner, Ulshafer, Wittbecker, Goldsmith and Ruof, *ibid.*, **69**, 2198 (1947).

(3) Ref. 2, p. 2201.

(4) Ref. 2, p. 2199.