There has recently been reported by Laland and Klem[12] the separation from liver of an amorphous, reddish-yellow material, in a yield of 0.35 mg. from 100 g. of fresh liver, with the following composition: C, 53.64; H, 6.85; N, 13.33; S, 0.74 (ash content 2.05%). The absorption spectrum in the ultraviolet showed two points of inflection, one between 2500 and 2650 Å. and another between 3450 and 3500 Å. The similarity between the nitrogen content and one point of inflection of the absorption spectrum of the material of Laland and Klem and fraction I is worthy of note. The material of Laland and Klem has been found to be therapeutically active, in doses of 0.7 mg., by Strandell, Poulsson and Schartum-Hansen.[13] Proof is still lacking that the chemically active material reported in this paper is a single pure chemical substance.

(12) Laland and Klem, *Acta Med. Scand.*, **88**, 620 (1936).

Summary

There is described the isolation from commercial liver extract of a microcrystalline white substance, as a sulfate, in a yield of 2 mg. from 100 g. of fresh liver, exhibiting intense blue fluorescence when exposed to ultraviolet light, and exerting therapeutic activity in pernicious anemia.

(13) Strandell, Poulsson and Schartum-Hansen, *ibid.*, **88**, 624 (1936).

BOSTON, MASS. RECEIVED AUGUST 19, 1936

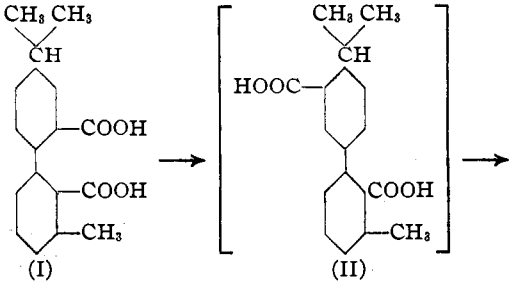
[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY]

Investigations in the Retene Field. VII. Certain Fluorenones and Phenanthridones from Retenediphenic Acid

BY DAVID E. ADELSON[1] AND MARSTON TAYLOR BOGERT

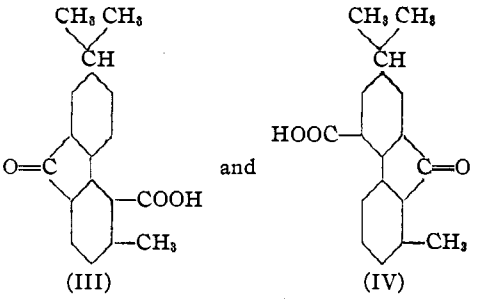
In a previous publication[2] it was shown that the oxidation of retenequinone in glacial acetic acid solution with 30% hydrogen peroxide yielded retenediphenic acid. This paper is concerned with the preparation and proof of structure of some additional derivatives of retenediphenic acid and with the establishment of the configuration of compounds previously reported.[2]

When retenediphenic acid (I) was treated with 95% sulfuric acid, a rotation of one of the rings of the biphenyl nucleus took place and the hypothetical intermediate (II) thus formed lost a molecule of water in two ways to yield 6-methyl-2-isopropylfluorenone-5-carboxylic acid (III) and 1-methyl-7-isopropylfluorenone-5-carboxylic acid (IV).



(1) Fritzsche Fellow in Organic Chemistry, Columbia University, 1935–1936.

(2) Adelson, Hasselstrom and Bogert, THIS JOURNAL, **58**, 871 (1936).

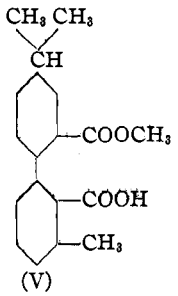


At 110° the principal product was the keto acid (III), while the isomeric acid (IV) was isolated when the reaction was carried out at room temperature. At 60° there resulted a mixture of acids which could not be separated by fractional crystallization; at 175° complete sulfonation took place. These keto acids, (III) and (IV), were characterized by conversion into the oximes, the methyl esters and the oximes of the latter.

At 110° the action of 95% sulfuric acid on the 3-methyl-4′-isopropyl-2′-carbomethoxybiphenyl-2-carboxylic acid (V) resulted in a mixture of the keto acids, (III) and (IV), and the methyl ester of (IV). This is similar to the experience of Underwood and Kochmann,[3] who studied the action of sulfuric acid on methyl acid diphenate. At room temperature, however, 95% sulfuric acid converted the acid ester (V) into the methyl ester

(3) Underwood and Kochmann, *ibid.*, **46**, 2074 (1924).

of (IV) in quantitative yield. The last reaction furnished the important link essential to the determination of the structures of the compounds reported in this and the previous paper.[2]



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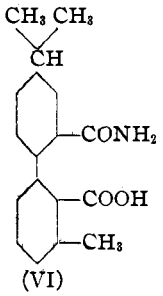
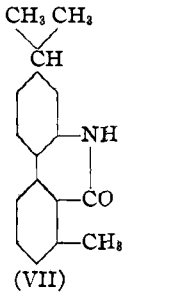
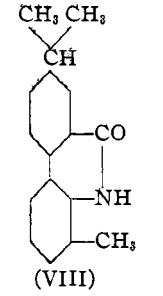


(VI)



(VII)



(VIII)

Concentrated ammonium hydroxide converted the acid methyl ester (V) into 6-methyl-5′-isopropyl-1′-diphenamic acid (VI) which yielded 8-methyl-2-isopropylphenanthridone (VII) when subjected to the Hofmann degradation. This phenanthridone (VII) was isomeric but not identical with the product obtained from the Beckmann rearrangement of 1-methyl-7-isopropylfluorenonoxime.[4] The previously reported phenanthridone is therefore 1-methyl-7-isopropylphenanthridone (VIII). Oxidation of (VII) yielded hemimellitic acid, thus indicating that the amide group in the amic acid (VI) and the ester group in the acid methyl ester (V) were in the same benzene nucleus as the isopropyl group. From this it followed that the action of 95% sulfuric acid on (V) at room temperature should yield the methyl ester of (IV) and that the acid (III), isomeric with (IV), should not yield retene ketone (1-methyl-7-isopropylfluorenone) upon decarboxylation, and such was actually found to be the case.

As a by-product in the action of 95% sulfuric acid on retenediphenic acid at 110°, there was formed a small amount of a neutral body. Its structure has not yet been determined.

Pyrolysis of retenediphenic acid yielded retene ketone.[5,6] This is analogous to the behavior of diphenic acid, which yielded fluorenone under similar treatment.[7,8] A better yield of retene ketone was obtained by dry distillation of retenediphenic anhydride.[2] Fluorenone has been obtained from diphenic anhydride in similar fashion.[8] The over-all yield of the ketone, based on retene, was 21%, whereas the older method of preparation[6] gave a yield of 13%.

So far as our experiments have gone, only one of the possible stereoisomers was encountered in these reactions.

Acknowledgments—We are indebted to Dr. Torsten Hasselstrom for preliminary work on the preparation of 6-methyl-2-isopropylfluorenone-5-carboxylic acid and for authentic samples of 1-methyl-7-isopropylphenanthridone and retene ketone.

Experimental

6 - Methyl - 2 - isopropylfluorenone - 5 - carboxylic Acid (III).—A solution of 10 g. of retenediphenic acid in 25 cc. of 95% sulfuric acid was maintained at 110–115° for twenty minutes, cooled and poured onto cracked ice. The yellow solid which this precipitated was dissolved in ether, and the solution washed with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded 9 g. of a mixture of keto acids, (III) and (IV). Because of the similarity of their solubilities in organic solvents, their separation proved very difficult. Fractional crystallization of the mixture from benzene gave 3 g. of the keto acid (III), which was then recrystallized from 90% formic acid, appearing as long, yellow needles, m. p. 156–156.5° (corr.).

Anal. Calcd. for $C_{18}H_{16}O_3$: C, 77.11; H, 5.76. Found: C, 76.78; H, 5.86.

Upon dry distillation of this acid, carbon dioxide was evolved and there appeared in the distillate a neutral, yellow oil, which could not be obtained in the crystalline state even when seeded with a crystal of retene ketone.

When the combined benzene mother liquors from the above operations was evaporated to dryness and the residue boiled with 10% sodium carbonate solution, there remained a small amount of undissolved material. This neutral body, which was also insoluble in hot dilute caustic alkali, appeared as small, yellow leaflets from 95% ethanol, m. p. 194–195° (corr.). Its structure has not yet been determined. Upon acidification of the carbonate filtrate, the mixture of acids was reprecipitated. Attempts to isolate the isomeric acid (IV) from such a mixture were unsuccessful.

Methyl Ester.—The dry potassium salt (1 g.) of the keto acid (III) was boiled with 5 g. of dimethyl sulfate for fifteen minutes, cooled and the excess dimethyl sulfate destroyed by pouring the reaction mixture into water. The resulting suspension was extracted with ether, the ethereal solution washed with 10% sodium carbonate solution, water and

(4) Bogert and Hasselstrom, THIS JOURNAL, **56**, 983 (1934).
(5) Bamberger and Hooker, *Ann.*, **229**, 135 (1885).
(6) Komppa and Fogelberg, THIS JOURNAL, **54**, 2900 (1932).
(7) Fittig and Obermeyer, *Ber.*, **5**, 933 (1872); *Ann.*, **166**, 372 (1873).
(8) Huntress, Hershberg and Cliff, THIS JOURNAL, **53**, 2720 (1931).

finally dried over anhydrous magnesium sulfate. Evaporation of the solvent, followed by recrystallization of the residual yellow solid (0.7 g.) from methanol, yielded tiny glistening flakes, m. p. 86.5–87.5° (corr.).

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.51; H, 6.17. Found: C, 77.37; H, 5.91.

Oxime of the Methyl Ester.—A suspension of 0.6 g. of the above methyl ester, 1 g. of hydroxylamine hydrochloride and 1.5 g. of anhydrous barium carbonate in 20 cc. of absolute methanol was refluxed for eight hours, filtered and the filtrate diluted with water. This precipitated the oxime in nearly theoretical yield. Recrystallized from dilute ethanol, it appeared as short, pale yellow needles, m. p. 173–174° (corr.).

Anal. Calcd. for $C_{19}H_{19}O_3N$: C, 73.75; H, 6.20. Found: C, 73.86; H, 5.92.

The oxime of the free acid, prepared similarly, appeared as short, cream-colored needles from dilute ethanol which darkened slightly over 250° and melted with decomposition at 268–269° (corr.).

Anal. Calcd. for $C_{18}H_{17}O_3N$: C, 73.19; H, 5.81. Found: C, 73.19; H, 6.09.

1 - Methyl - 7 - isopropylfluorenone - 5 - carboxylic Acid (IV).—Retenediphenic acid (2.5 g.) was dissolved in 10 cc. of 95% sulfuric acid at room temperature and, after twenty-four hours, the clear solution was poured onto cracked ice. The yellow precipitate thus formed was filtered, washed with water and recrystallized from 90% formic acid, from which it appeared as broad, yellow leaflets, m. p. 200.5–201° (corr.); yield 1.0 g.

Anal. Calcd. for $C_{18}H_{16}O_3$: C, 77.11; H, 5.76. Found: C, 76.62; H, 5.94.

When retenediphenic acid was heated with 95% sulfuric acid at 60° for twenty minutes and the reaction mixture worked up as described for the above keto acid (III), a mixture of products resulted which could not be separated by fractional crystallization. At 175° complete sulfonation resulted even when heat was applied for only two minutes.

Methyl Ester.—This was prepared from the potassium salt of the keto acid (IV) in a manner similar to the synthesis described above for the isomeric methyl ester. Recrystallized from methanol, it appeared in tufts of short, bright yellow needles, m. p. 104–104.5° (corr.).

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.51; H, 6.17. Found: C, 77.31; H, 6.27.

Two grams of the acid methyl ester (V) was dissolved in 8 cc. of 95% sulfuric acid at room temperature and, after a day, the crimson solution was poured into water, a yellow precipitate forming. This was dissolved in ether, the ethereal solution was washed with 10% sodium carbonate solution, water and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave the keto methyl ester in practically quantitative yield; recrystallized from methanol, it appeared as tufts of short, bright yellow needles, m. p. 104–104.5° (corr.). Mixed with a sample of the above methyl ester of m. p. 104–104.5° (corr.), it gave no depression.

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.51; H, 6.17. Found: C, 77.34; H, 6.45.

The oxime of the methyl ester, prepared similarly to the isomeric oxime described above and crystallized from dilute ethanol, appeared as clusters of long, cream-colored needles, m. p. 151.5–152° (corr.).

Anal. Calcd. for $C_{19}H_{19}O_3N$: C, 73.75; H, 6.20. Found: C, 73.72; H, 6.52.

The oxime of the keto acid (IV), prepared similarly, crystallized from dilute ethanol in short, pale yellow needles which melted with decomposition at 249–250° (corr.).

Anal. Calcd. for $C_{18}H_{17}O_3N$: C, 73.19; H, 5.81. Found: C, 73.12; H, 5.78.

6 - Methyl - 5′ - isopropyl - 1′ - diphenamic Acid (VI).—The acid methyl ester (V) was dissolved in concentrated ammonium hydroxide and allowed to stand with occasional shaking for a week. Dilution of the solution followed by acidification yielded a white precipitate which appeared as colorless, hard prisms (yield 25%) from 95% ethanol, m. p. 197–198° (corr.). Mixed with an authentic specimen of the amic acid (VI) of m. p. 201–202° (corr.), it melted at 200–201° (corr.).

8-Methyl-2-isopropylphenanthridone (VII).—To a solution of 3.5 g. of the amic acid (VI) in 30 cc. of 10% potassium hydroxide, there was added slowly a solution of 3 g. of bromine in 30 cc. of 10% potassium hydroxide. The clear solution was cooled in an ice-bath for thirty minutes, heated at 60–65° for another thirty minutes, cooled, 1 g. of sodium bisulfite added and the resulting solution acidified with concentrated hydrochloric acid. The voluminous white precipitate was boiled with 50 cc. of 5% potassium hydroxide solution, filtered, washed with water, dried at 110°, and recrystallized from benzene, 95% ethanol and finally from benzene, from which it appeared as long, silky white needles, m. p. 230–231° (corr.). A mixture of this and an authentic specimen of 1-methyl-7-isopropylphenanthridone[4] (VIII) of m. p. 221–222.5° (corr.) melted at 187–188° (corr.).

Anal. Calcd. for $C_{17}H_{17}ON$: C, 81.23; H, 6.82. (Calcd. for the amino carboxylic acid, $C_{17}H_{19}O_2N$: C, 75.79; H, 7.12). Found: C, 81.17; H, 7.10.

Two grams of the above phenanthridone was dissolved in 7 cc. of 95% sulfuric acid and the solution poured into 150 cc. of water. The finely-divided suspension was made alkaline with 10% potassium hydroxide, diluted to a volume of 400 cc., 17 g. of potassium permanganate added and the mixture was stirred and refluxed for twenty hours. The solution was filtered, the colorless filtrate evaporated to a small volume, cooled, extracted with 350 cc. of ether and the latter dried with anhydrous magnesium sulfate. Evaporation of the ether yielded 0.7 g. of a white solid which appeared as white tablets from an ether–petroleum ether mixture. After drying at 110° it melted at 188–189° (corr.), with decomposition. Upon cooling, the fused material solidified and melted at 196–197° (corr.). The recorded melting points for hemimellitic acid and hemimellitic anhydride[9] are 190 and 196°, respectively. The acid gave an excellent fluorescein test on fusion with resorcinol and sulfuric acid. Upon treating an aqueous solution of the acid with a concentrated potassium chloride solution, there resulted tiny, glistening flakes which are characteristic of the mono-potassium salt of hemimellitic acid.[9]

(9) Graebe and Leonhardt, *Ann.*, **290**, 220 (1896).

Anal. Calcd. for $C_9H_6O_6$: C, 51.42; H, 2.88. Found: C, 51.36; H, 2.97.

Retene Ketone (1-Methyl-7-isopropylfluorenone).—Two grams of retenediphenic anhydride was dry distilled, yellow vapors coming over accompanied by carbon dioxide. The distillate soon solidified to a yellow, crystalline material; yield, 1.5 g. (90%). From 95% ethanol it appeared as long, flat, sulfur-yellow prisms, m. p. 89–90° (corr.). Mixed with an equal amount of an authentic specimen of retene ketone of m. p. 88–89° (corr.), it melted at 89–90° (corr.).

When retenediphenic acid or an intimate mixture of its anhydride and soda lime were distilled, retene ketone also resulted. In these cases, however, losses due to charring were appreciable and the yields lower.

Summary

1. Dehydration of retenediphenic acid with 95% sulfuric acid yields two isomeric methylisopropylfluorenone carboxylic acids.

2. These keto acids have been characterized by their oximes, methyl esters and the oximes of the latter.

3. Ammonia converts methyl acid retenediphenate into the previously reported retenediphenamic acid which undergoes the Hofmann degradation to yield 8-methyl-2-isopropylphenanthridone. Oxidation of the latter yields hemimellitic acid, thus establishing the structures of the compounds concerned.

4. Treatment of methyl acid retenediphenate with 95% sulfuric acid at room temperature yields the methyl ester of 1-methyl-7-isopropylfluorenone-5-carboxylic acid. The isomeric acid is therefore 6-methyl-2-isopropylfluorenone-5-carboxylic acid.

5. Pyrolysis of retenediphenic anhydride gives retene ketone in 90% yield.

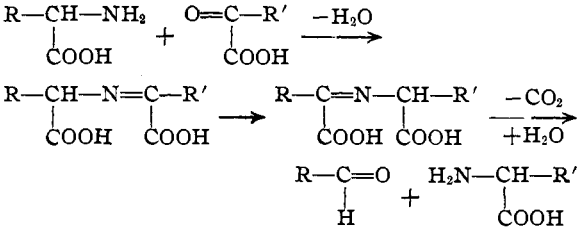
NEW YORK, N. Y. RECEIVED AUGUST 4, 1936

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, COLUMBIA UNIVERSITY]

The Reaction between α-Ketonic Acids and α-Amino Acids

BY ROBERT M. HERBST

In a previous paper[1] it was shown that a reaction takes place between certain α-ketonic acids and α-amino acids. The first step in the reaction was assumed to be interaction between the carbonyl and the amino groups to form a Schiff base. The second step involved the migration of a hydrogen atom from the α-carbon of the amino acid to the α-carbon of the ketonic acid residue, and the third the elimination of carbon dioxide from the intermediate and the addition of water resulting in fission with the formation of an aldehyde and an amino acid.



Certain results reported in the previous paper made it desirable to continue the investigation in order to gain greater insight into the mechanism of the reaction. In experiments with *l*-cystine and pyruvic acid acetaldehyde was always obtained in appreciable amounts. In the absence of any product, other than carbon dioxide, which could be attributed to the cystine molecule, it was concluded that the acetaldehyde was derived in some way from this source. This view has now been shown to be incorrect, for in the reaction between cystine and both phenylpyruvic and benzoylformic acids *no* acetaldehyde could be found. Moreover, small amounts of acetaldehyde have been isolated from the products of the reaction of pyruvic acid with *p*-methoxyphenylalanine, α-amino-*p*-methoxyphenylacetic acid, ethylcysteine, benzylcysteine and phenylcysteine, while with α-aminophenylacetic acid *no* acetaldehyde could be detected. These results point to pyruvic acid or some intermediate formed with the above amino acids as the principal source of acetaldehyde. The possibility remains that acetaldehyde could be formed by the secondary interaction of pyruvic acid with alanine formed during the primary reaction. In a separate experiment with this pair acetaldehyde was formed, but too slowly to account for its rapid formation in the reaction of pyruvic acid with cystine, S-phenylcysteine and S-benzylcysteine. The conclusion is therefore unescapable that the formation of acetaldehyde is largely a direct result of the primary reaction of pyruvic acid with the above amino acids.

(1) Herbst and Engel, *J. Biol. Chem.*, **107**, 505 (1934).