180°, the resulting base is purified through recrystallization of its picrate (A) from benzene and glacial acetic acid. The assigned structure, 3-methyl-8-ethylquinoline, was confirmed by synthesis.[9]

Synthesis of 3-Methyl-8-ethylquinoline.—Dry hydrogen chloride is introduced at a moderate rate into a mixture of propionic aldehyde (5 g.) and methylal (6 g.) for five minutes. On the addition of *o*-ethylaniline (1.8 g.) in concentrated hydrochloric acid (5 g.), a reaction begins and is completed by boiling the solution for three hours. An equal volume of water is added, the filtered solution is treated with a slight excess of sodium nitrite, and, after concentration to a small volume, the bases are liberated with sodium hydroxide and extracted with ether. Final purification of the methylethylquinoline, after fractional distillation at diminished pressure, is effected through the picrate (B). The identity of salts A and B was confirmed by a mixed melting point determination. The new base which was obtained in a yield of 8% has the following physical constants: b. p. 263° with partial decomposition (at 746 mm.); $n^{25}\text{D}$ 1.5946.

Anal. Calcd. for $C_{18}H_{16}O_7N_4$: C, 54.00; H, 4.00; N, 14.00. *Picrate A.* Found: C, 53.84; H, 4.07; N, 14.02. *Picrate B.* Found: C, 54.01; H, 4.00; N, 14.03.

Synthesis of 2,3-Dimethyl-8-ethylquinoline.—Final confirmation of the structure of the original kero base was effected through its synthesis[10] which can be carried out as follows: a mixture of concentrated hydrochloric acid (4 moles), tiglic aldehyde (1 mole) and *o*-ethylaniline hydrochloride (4 moles) is heated for four hours on a water-bath and then steam distilled. The solution, after liberation of the bases with sodium hydroxide, is again steam distilled and the distillate, acidified with hydrochloric acid, is treated at room temperature with sodium nitrite. Following the removal of the supernatant oil by ether extraction, the solution is boiled, filtered, made alkaline with sodium hydroxide, steam distilled and again extracted with ether. The base, regained from the solvent, gave a picrate which, mixed with the kero base picrate, produced no depression of the melting point (220°).

Anal. Calcd. for $C_{19}H_{18}O_7N_4$: C, 55.07; H, 4.35; N, 13.53. Found: C, 55.19; H, 4.27; N, 13.46.

Summary

The isolation of 2,3-dimethyl-8-ethylquinoline (I) from the complex mixture of bases obtained from the kerosene distillate of California asphalt-base petroleum is described. The structure assigned this product has been confirmed by synthesis.

2,3-Dimethylquinoline (II) and 3-methyl-8-ethylquinoline (III) were obtained by indirect dealkylation of base I at positions 8 and 2, respectively. Base II is one of five kero quinolines which were isolated in the Texas Laboratory prior to the present investigation. The structure of base III, a new quinoline homolog, was established by synthesis.

AUSTIN, TEXAS RECEIVED NOVEMBER 26, 1937

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[9] *Cf.* Carl Beyer, *J. prakt. Chem.*, **33**, 419 (1886); von Miller and Kinkelin, *Ber.*, **20**, 1916 (1887).

[10] *Cf.* Doebner and von Miller, *ibid.*, **16**, 2464 (1883).

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE WASHINGTON SQUARE COLLEGE, NEW YORK UNIVERSITY]

Change in the Optical Rotation of Gluconontrile

BY PHILIPPOS E. PAPADAKIS AND HAROLD JEROME COHEN

Geza Zemplén[1] prepared gluconontrile, m. p. 145° and $[\alpha]^{21}\text{D}$ +8.8, by hydrolyzing pentaacetyl gluconontrile. He found that by recrystallizing the product from absolute alcohol he could get gluconontrile melting at 115–120°. A. Wohl and O. Wollenberg[2] prepared gluconontrile from glucose and hydroxylamine acetate. The product was washed with different mixtures of acetic acid and acetone and further with acetone and finally with absolute ether. Wohl and Wollenberg did not give the specific rotation of their product. They repeated Zemplén's work and state: "Remarkably also a melting point of 145° and a melting point of the recrystallized substance of 115–120° is reported. By the reported procedure of recrystallization from absolute alcohol as well as by the wash procedure, as was described above, a melting point of 146–148° is found and the substance gives no depression with that produced in the above described manner."

As far as we are aware no further investigation has been made to find out whether Zemplén's observations were correct. As the problem seemed interesting, it was thought advisable to prepare gluconontrile by the Wohl and Wollenberg method and then recrystallize it from absolute ethyl alcohol to find out whether gluconontrile, m. p. 115–120°, could be obtained. If the observations of Zemplén are correct, then both the high and the low melting point glucononi-

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[1] G. Zemplén, *Ber.*, **60**, 171 (1927).

[2] A. Wohl and O. Wollenberg, *Ann.*, **500**, 281 (1932).

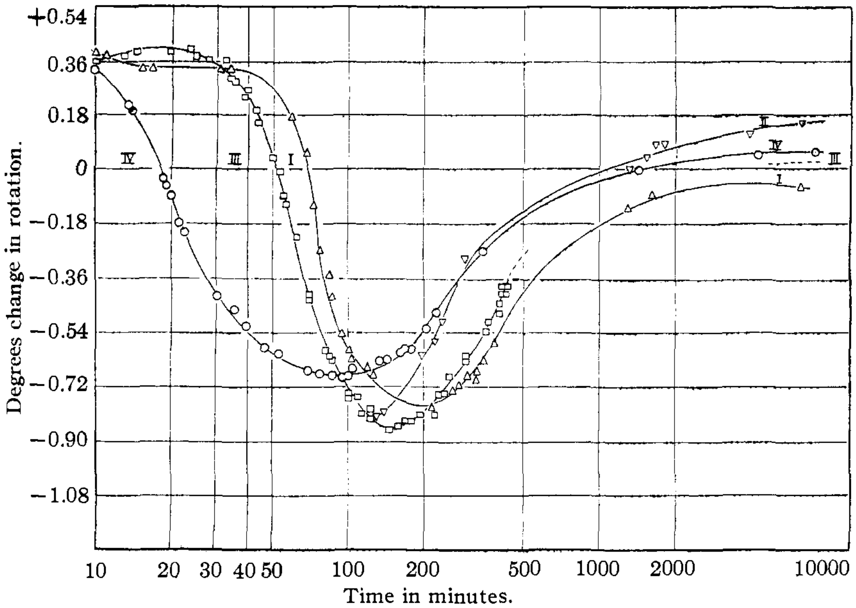


Fig. 1.—Preparation Ia: Curve I, room temperature 25°, 4.800 g. per 100 cc. water solution; Curve II, thermostat 31°, room temperature 24°, 4.760 g. per 100 cc. water solution. Preparation Ib: Curve III, thermostat 25°, room temperature 24.5°, 4.744 g. per 100 cc. water solution. Preparation Ic: Curve IV, thermostat 25°, room temperature 23–24°, 0.5840 g. + 15 cc. water.

trile should be purified further and then examined in as many ways as possible.

By repeating Wohl and Wollenberg's work gluconitrile, m. p. 148°, was obtained. Then by recrystallizing it four times from glacial acetic acid a product resulted which melted at 145°; on slight further heating the liquid began to rise in the capillary. The specific rotation in water solution was $[\alpha]^{24}_D$ +9.96° (0.4391 g. in 25 cc. of water solution in a 1-dm. tube rotates 0.175° to the right). The rotation remained constant for at least three days of observations. The specific rotation in pyridine solution was $[\alpha]^{24}_D$ +6.27° (0.1196 g. in 2.5 cc. pyridine solution in a 0.5-dm. tube rotates 0.15° to the right).

Anal. Calcd. for $C_6H_{11}O_5N$: C, 40.66; H, 6.10; N, 7.90. Found: C, 40.62; H, 6.28; N, 7.96.

By recrystallizing some of the original material of m. p. 148° three times from absolute alcohol, then by redissolving in hot absolute alcohol, bone-blacking, filtering and recrystallizing, a product was obtained which melted at 120.5°; on further heating it semi-solidified and at 145° melted and the liquid began to rise in the capillary. The specific rotation in water solution at zero time was $[\alpha]^{25}_D$ +10.00° (0.1200 g. in 2.5 cc. of water solution in a 0.5-dm. tube rotates 0.24° to the right). The rotation 0.24° is extrapolated from the curve obtainable by plotting the results of the first few observations, which are not shown in Curve I. The specific rotation on the basis of the first observation, taken eight minutes after the zero time, was $[\alpha]^{25}_D$ +8.75°. Zemplén found 8.8°. The specific rotation in pyridine was $[\alpha]^{24}_D$ +6.03° (0.1160 g. in 2.5 cc. of pyridine solution in a 0.5-dm. tube rotates 0.14° to the right).

Anal. Calcd. for $C_6H_{11}O_5N$: C, 40.66; H, 6.10; N, 7.90. Found: C, 40.72; H, 6.32; N, 7.92.

The high melting point, 145°, and the low melting point, 120.5°, as found above confirm Zemplén's observations. Mixed melting points of a 1:1 mixture of the sample melting at 145° and the sample melting at 120.5° melted at 131°. Mixed melting points of different preparations having melting points between 120.5 and 145° were approximately the average of their respective melting points.

Gluconnamed, m. p. 145°, when dissolved in water, had a constant rotation, whereas the rotation of glucononitrile, m. p. 120.5°, underwent a complex change as is shown in Curves I to IV, Fig. 1. The rotation of the latter started positive, diminished, remained constant for a while and then decreased, reached a minimum and then it rose slowly and finally became positive again. As the course of the change studied was not strictly at constant temperature, the rate of change shown was only approximate.

The experiment under Preparation Ib, Curve III, was run as follows. The solution was made at 25° and a 0.5-dm. semimicro tube was filled and kept in a thermostat at 25°. To observe the change in rotation the tube was withdrawn at intervals, dried quickly and the readings were taken at room temperature, 24.5°. Similarly in all other cases the observations were taken quickly at room temperature and then the polariscope tube was replaced in the thermostat. A 0.5-dm. semimicro polariscope tube was used in all experiments except those in Curve IV where a 1-dm. tube was employed. To obtain comparable results all the values in the curves have been plotted on the basis of a 1-dm. tube.

From curves I–III, it appears that more than one type of change may be involved in the change of rotation of glucononitrile, m. p. 120.5°. Whether the low melting point glucononitrile is a labile form or whether the changes it undergoes in water solution are caused by the catalytic effect of some extraneous substance or by a change in condition, has not been determined as yet. With the object of eliminating, if possible, traces of such extraneous material, the high melting point glucononitrile was changed to the one of low melting point and the low to the high by changing solvents, as the diagram shows.

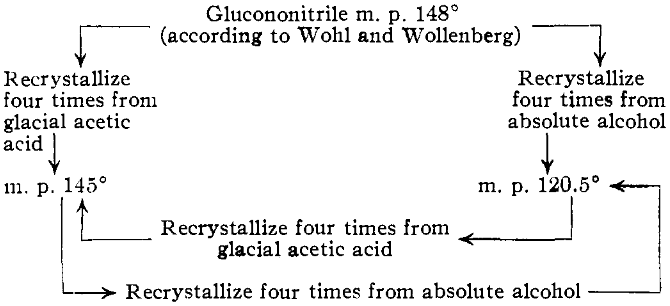
Glucononitrile, m. p. 145°, was recrystallized from absolute alcohol four times. The resulting crystals melted at 120.5°. A sample of this preparation Ic was used to find out whether it would give results similar to Preparations Ia and Ib, Curves I to III, which were prepared directly from the crude material by recrystallizing from absolute alcohol.

COMPARATIVE TABLE I

GLUCONONITRILE IN WATER SOLUTION

Preparation and curve	Concn., %	Length of tube, dm.	Rotation at beginning	Minimum rotation	Rotation of last observation	Specific rotation at beginning	Specific rotation at minimum	Spec. rotation at last observation
Ia Curve I	4.800	0.5	+ 0.24 x + .21 f. o.	−0.40	−0.03 n. f.	+10.00 + 8.75	−16.66	−1.24
Ia Curve II	4.760	.5		− .41	+ .08 n. f.		−17.22	+3.36
Ib Curve III	4.744	.5	+ .23 f. o.	− .43	+ .05 n. f.	+ 9.69	−18.11	+2.10
Ic Curve IV	3.893 g. + 100 cc. H_2O	1	+ .39 x + .36 f. o.	− .63	+ .06 n. f.	+10.02 n. c. v. + 9.25 n. c. v.	−16.18 n. c. v.	+1.55
II	1.7564	1	+ .175		+ .175	+ 9.96		+9.96

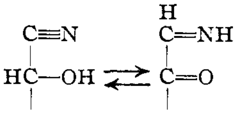
Remarks: x = extrapolated; f. o. = first observation; n. f. = not final, rotation still changing; n. c. v. = not corrected for volume change: only approximate. Preparation Ia, m. p. 119; Ib and Ic, m. p. 120.5; II, m. p. 145°.



The results in Curve IV show that in a general way Preparation Ic undergoes changes similar to Ia and Ib, with this exception, that the constancy in rotation observed a few minutes after the beginning of the experiments with Ia and Ib is eliminated. It is observed also that if the degrees of rotation in Curve IV be multiplied by a factor so as to get concentration comparable with Preparation Ib, Curve III, the minimum of the curve is approximately the same. The specific rotation of Preparation Ic in water solution was $[\alpha]^{25}D$ +10.02° (solution of 0.5840 g. + 15 cc. water, placed in 1-dm. tube rotates 0.39° to the right). The value 0.39° is extrapolated from the curve obtainable by plotting the results of the first few observations which unfortunately are not shown in Curve IV. The specific rotation on the basis of the first observation was $[\alpha]^{25}D$ +9.25°. It is observed here that the extrapolated value for the specific rotation of Preparation Ic was the same as that of the high melting substance.

Gluconónitrile, m. p. 120.5°, from the Preparation Ib was recrystallized from glacial acetic acid four times. The resulting substance melted at 145°. When dissolved in water the optical rotation remained constant for at least two days.

The products finally obtained upon evaporating the water from the gluconónitrile solutions used in the long-time experiments have not yet been analyzed. It has been observed that crystals can be regained much more easily from the solution containing the gluconónitrile of the higher melting point. Although it is difficult, at present, to give a satisfactory explanation of the processes involved in the change of rotation of gluconónitrile, m. p. 120.5°, in water solution, it is considered possible that if mutarotation takes place, besides other subsequent reactions, it will give rise to *cis* and *trans* isomerism. It is also thought that if a change could take place in the loading of carbon one and two in the following manner



$$\begin{array}{c} C{\equiv}N \\ | \\ HC{-}OH \\ | \end{array} \rightleftarrows \begin{array}{c} H \\ C{=}NH \\ | \\ C{=}O \\ | \end{array}$$

it would give rise to an imino group[3,4] as well as a carbonyl group, each one of which might mutarotate. In the hope of arriving at a plausible explanation for the unusual change in rotation, the Raman and absorption spectra of the solutions will be measured and these results will furnish the basis for a further communication.

The authors wish to express their thanks to Mr. D. Rigakos of the Rockefeller Institute for Medical Research for the microanalytical work.

Summary

1. Gluconónitrile, recrystallized from acetic acid, melted at 145°. Gluconónitrile, recrystallized from absolute alcohol, melted at 120.5°. These results confirm Zemplén's observations.

2. Mixed melting points of a 1:1 mixture of the two forms gave as the melting point approximately the average of the respective melting points.

3. Gluconónitrile, m. p. 145°, had a specific rotation in water solution of +9.96°. The optical rotation remained constant.

4. Gluconónitrile, m. p. 120.5°, had a specific rotation in water solution of approximately +10.00 at initial time. The optical rotation changed from positive to negative, reached a minimum, then reversed its course and slowly became positive again.

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[3] J. C. Irvine, R. F. Thomson and C. S. Garrett, *J. Chem. Soc.*, **103**, 239 (1913).

[4] P. Levene, *J. Biol. Chem.*, **24**, 60 (1916).

5. The specific rotation of both the low and high melting point gluconitriles in pyridine was practically the same, +6.03° and +6.27°, and constant.

6. When gluconitrile, m. p. 120.5°, was recrystallized four times from acetic acid, gluconitrile of m. p. 145° was obtained. The latter in water solution had constant rotation.

7. When gluconitrile, m. p. 145°, was recrystallized four times from absolute alcohol, gluconitrile of m. p. 120.5° resulted. The latter in water solution showed change in optical rotation. In the authors' knowledge no such unusual reversal of rotation has been observed before in mutarotating sugars.

NEW YORK, N. Y. RECEIVED JANUARY 26, 1938

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, COLLEGE OF ST. TERESA]

Chemical Studies of the Dissociants of the H-37 Human Tubercle Bacillus[1]

BY GUSTAV J. MARTIN

In recent years a large number of papers have appeared describing polymorphism and morphological instability in the etiological agent of tuberculosis. The dissociation of *Mycobacterium tuberculosis* into virulent S and avirulent R types by Petroff[2] changed the aspect of chemical investigation of that organism. It was deemed possible that the predominance of one form in any culture might entirely change the outcome of an experiment. Cooper[3] undertook the investigation of the R and S forms of the bacillus of Calmette-Guerin. He encountered insurmountable technical difficulties, but by treating each type in exactly the same manner he was able to get comparative if not quantitative values. The R or avirulent type produced more lipid than the S or virulent type. Differences were found also in the culture medium after growth, the S type elaborating a greater quantity of carbohydrates and soluble substances in general than the R type.

The objective of this preliminary work on the chemistry of the variants of the human (H-37) tubercle bacillus was to subject both to exactly the same treatment and attempt an estimation of quantitative differences. It is our working hypothesis that virulence is a quantitative factor and not a qualitative factor, as is commonly held. Virulence is probably dependent on the presence in the virulent variant of a larger percentage of a certain component. The two variants used, designated as Rv (virulent) and Ra (avirulent), were isolated several years ago and have maintained their biological and morphological characteristics throughout this period. Transplants of stabilized cultures of the two variants were made on the synthetic media used successfully by Petroff. Asparagin is the sole source of nitrogen in this medium. Its composition is as follows

KH_2PO_4, g.	5.0
$MgSO_4$ anhyd., g.	0.6
Mg citrate, g.	2.5
Asparagin, g.	5.0
Glycerol, cc.	20

Dilute to 1 liter with distilled water.

A luxuriant growth appeared in six weeks at 37.5°. The bacteria were then separated from the liquid medium by filtration first through paper and then through Berkefeld candles. After thorough washing, the bacterial residues were dried *in vacuo* over sulfuric acid to constant weight. The bacteria were then placed in thimbles and the Soxhlet extractions started. The entire procedure from initial separation of the bacteria from the media, to the final extractions, was carried out under carbon dioxide. Freshly distilled organic solvents were used throughout. Specially designed covers for the thimbles were added to the ordinary Soxhlet extraction set-up to prevent spattering of bacilli over the sides of the thimble. The extractions were continuous for eight hours per day, and for six days a week. The extraction with each solvent was continued until no further detectable quantities of lipoid appeared. As indicated in Table I, the initial extractions were made in Soxhlet extractors using ether as the solvent. This continued over a six-week period. A second Soxhlet extraction was made with acetone and was continued for a second six-week period. Next, the bacteria which previously had been extracted with both ether and acetone were treated with boiling chloroform for a period of three weeks (eight hours daily). Finally, a reëxtraction with the solvents acetone and chloroform was made, using the liquids at the boiling point. This reëxtraction was motivated by the work of Hecht,[4] who has presented evidence indicating the presence of sterols in the tubercle bacillus.

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[1] This study was made possible by a grant from the Committee on Scientific Research of the American Medical Association.

Bacteria grown by William Steenken, Jr., were contributed by the Trudeau Foundation, Trudeau, N. Y.

[2] S. A. Petroff, *Proc. Soc. Exptl. Biol. Med.*, **24**, 632 (1927).

[3] Frank B. Cooper, *J. Biol. Chem.*, **88**, 485 (1930).

[4] Eugen Hecht, *Z. physiol. Chem.*, **231**, 29, 279 (1935).