of rats is a hybrid one, predominately Wistar. The Denver University strain is a hybrid which has been inbred at the local colony for many years. The Yale strain used was a pure line obtained from the University of Colorado School of Medicine.

CONCLUSIONS

1. Mixing weighed quantities of red squill powder with food to make 1 per cent body weight bioassay baits is not satisfactory for accurate studies, since the ratio of poison to food will vary, and a definite food interference is noted when small doses of squill are fed.

2. It is satisfactory either to weigh out both the red squill powder and the bait for the bioassay bait and mix for each individual test animal, or previously to blend a standard concentration bait and weigh the proper quantities of this food to give the doses desired.

3. The percentage of red squill powder in the bioassay bait is of importance, both as regards the ultimate toxicity and the speed of action of the poison.

4. The strain of rat used does not affect the bioassay, when the animals have been maintained on the same diet for at least a week before the beginning of the bioassay. The rats used should be of approximately the same age when bioassays on two or more strains are to be carefully compared.

5. Depending upon the quality of the squill powder being tested, from 0 to $33^1/_3$ per cent of the animals survive longer than three days, although they ultimately die with typical red squill symptoms, and accordingly must be counted in the bioassay. In many cases the tests must be allowed to continue for seven or eight days.

6. Female rats are more than twice as susceptible to red squill powder as are male rats.

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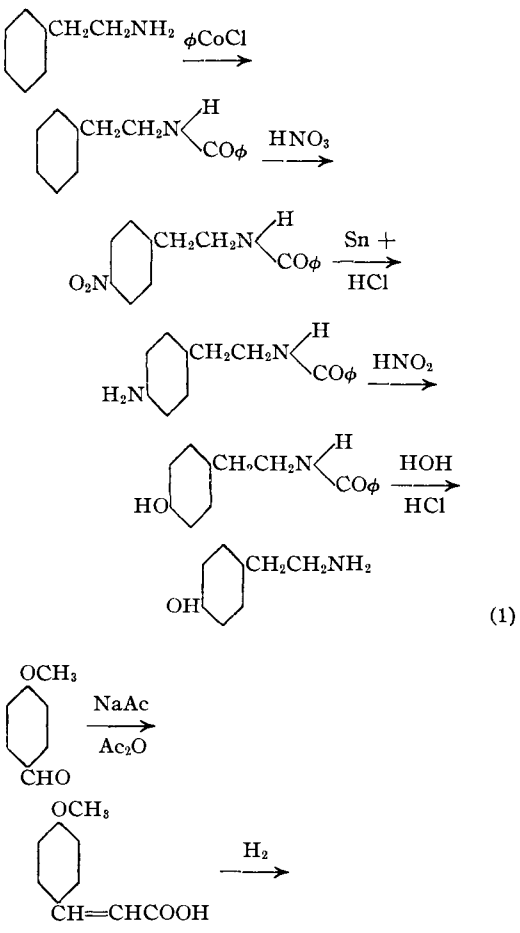
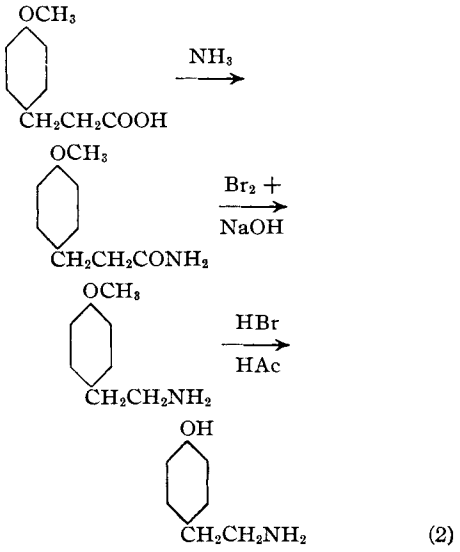
The Synthesis of Hydroxymandelonitrile Dibenzoates

By Kenneth E. Hamlin, Jr., and Walter H. Hartung*

The biological importance of tyramine and the rather incompletely reported pharmacological studies of other β-hydroxyphenylethylamines led to the investigation of possible intermediates for a synthesis of tyramine and its isomers. Previous syntheses have been reported by Barger and Walpole (1) from phenylethylamine and from anisaldehyde as indicated by:

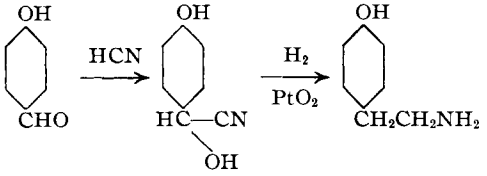


* University of Maryland Alumni Fellow, 1938–1939; Wm. R. Warner Fellow 1939–1940.



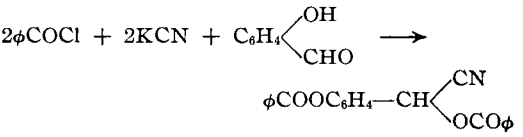
(2)

Buck (2) reports a new synthesis of tyramine through the reduction of hydroxymandelonitriles. His preparation of *p*-hydroxymandelonitrile from the corresponding hydroxybenzaldehyde in 38% yields and its subsequent reduction to tyramine in 48% yields (the overall yield being less than 20%) can be represented thus:



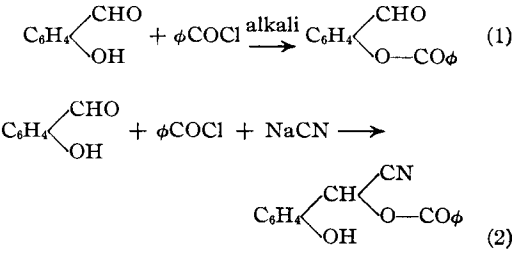
It has been established by Francis and Davis (3) that benzaldehyde in the presence of potassium cyanide and benzoyl chloride readily forms the benzoate of mandelonitrile, $\phi\text{—CH(CN)—O—CO}\phi$, ($\phi$ = C_6H_5) in good yields.

Hartung (4) has described the catalytic reduction of mandelonitrile to β-phenylethylamine. A combination of these reactions suggested a synthesis of tyramine and its isomers. The hydroxybenzaldehydes, however, because of their phenolic character and the basic nature of the alkali cyanides, simultaneously undergo the Schotten-Bauman reaction, thereby complicating results. Aloy and Rabaut (5) described the preparation of *p*- and *o*-hydroxymandelonitrile dibenzoates in the following way:

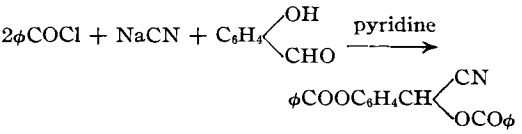


They reported no yields and only the para-compound was analyzed. The ortho- isomer was described as a liquid; no reference was made to the *m*-hydroxymandelonitrile dibenzoate.

Since here are two competing reactions, *viz.*,



it is obvious that the ultimate results will be influenced by the relative speeds of these reactions. Consequently if one can first be completed, the second ought to proceed with a minimum of interference. For example, the esterification of the phenolic hydroxyl may be carried out with benzoyl chloride using a tertiary organic base such as pyridine, and then the cyanhydrin formation with accompanying esterification takes place without interference. Thus, the benzoates of the hydroxybenzaldehydes give quite as satisfactory yields of the cyanhydrin benzoate as does benzaldehyde itself. In practice, however, it is unnecessary first to isolate the ester of the phenolic aldehyde, for it is possible to esterify the hydroxyl group and simultaneously obtain the benzoyl derivative of the cyanhydrin as indicated by:



Thus far, it has been possible to obtain yields up to 67% of *p*-hydroxymandelonitrile dibenzoate by this reaction on *p*-hydroxybenzaldehyde and 45% of *o*-hydroxymandelonitrile dibenzoate from *o*-hydroxybenzaldehyde. Because of the scarcity of *m*-hydroxybenzaldehyde, it has been impossible

to obtain the optimum conditions for the best yields of the *m*-hydroxymandelonitrile dibenzoate. Contrary to the work of Aloy and Rabaut, the *o*-hydroxymandelonitrile dibenzoate was purified as a crystalline solid.

Since these derivatives, which are all stable, crystalline solids, melting without decomposition, may be synthesized with comparative ease, the course of their reduction merits attention. In the light of the previous work, catalytic reduction is expected to proceed according to the following equation:

$$\phi COOC_6H_4CH\begin{matrix}CN\\OCO\phi\end{matrix} \xrightarrow[\text{simultaneous alcoholysis}]{H_2\,(Pd)} HO\cdot C_6H_4\cdot CH_2CH_2NH_2 + 2\phi COOC_2H_5$$

Furthermore, if the reduction can be influenced to take the following course,

$$\phi COOC_6H_4CH\begin{matrix}CN\\OCO\phi\end{matrix} \xrightarrow[\text{simultaneous alcoholysis}]{H_2(Pd)} HO\cdot C_6H_4\cdot CH\begin{matrix}CH_2NH_2\\O{-}CO\phi\end{matrix} + \phi COOC_2H_5$$

the product, $HOC_6H_4CH\begin{matrix}CH_2NH_2\\O{-}CO\phi\end{matrix}$, will prove of unusual interest because in this single molecule are combined the optimum structural elements for pressor activity and a potent anesthesiophore group.

EXPERIMENTAL

Hydroxybenzaldehyde Benzoates.—The preparation of the hydroxybenzaldehyde benzoates is carried out using pyridine as the base in the benzoylation reaction. A slight excess of benzoyl chloride is added to molecular quantities of the hydroxybenzaldehyde and pyridine. Considerable heat is generated and reaction is immediate, but usually the mixture if allowed to stand over night gives better yields. The solution is extracted with ether and washed thoroughly with dilute hydrochloric acid to remove the pyridine and with dilute alkali to remove the excess benzoyl chloride. After removal of the ether, the isomeric hydroxybenzaldehyde benzoates are purified as described below.

The *p*-hydroxybenzaldehyde benzoate is a solid and can be recrystallized from alcohol. On precipitation from ether using petroleum ether, white crystals are obtained as long fine needles, melting from 90.0–90.5°.[1] The phenylhydrazone of *p*-hydroxybenzaldehyde benzoate recrystallized from alcohol gives white, fluffy needles, melting at 173–174°.

The *o*-hydroxybenzaldehyde benzoate is a viscous, water-white liquid, distilling from 184–185° at 2-mm. pressure. Vavon (6) has previously reported the boiling point as 207–208° at 13 mm. The phenylhydrazone from alcohol gives small yellow needles melting at 137–138°.

The *m*-hydroxybenzaldehyde benzoate is a white crystalline compound. On recrystallization from alcohol as fine needles, it melts from 48.5–49.0°. Previous reference to this compound could not be found in the literature.

Hydroxymandelonitrile Dibenzoates.—The hydroxymandelonitrile dibenzoates are prepared from the hydroxybenzaldehyde benzoates in the following way. To molecular quantities of the hydroxybenzaldehyde benzoate and pyridine is added a slight excess of sodium cyanide (as a saturated aqueous solution). On shaking and cooling, the corresponding cyanhydrin is formed as a solid compound. Next, an equivalent quantity of benzoyl chloride is added slowly in small portions. The mixture becomes highly colored and much heat is produced, constant cooling and shaking being necessary. After reaction, if dilute hydrochloric acid is added to the mixture, an oil separates out which solidifies on standing. The solid matter is filtered off and recrystallized from a suitable solvent. Some difficulty was experienced in selecting the proper solvent for purification of each solid isomer.

It was found that if the proper hydroxybenzaldehyde is dissolved in two equivalents of pyridine, a slight excess of sodium cyanide (as the saturated aqueous solution) is added and then two equivalents of benzoyl chloride are added slowly, with constant shaking and cooling, the identical hydroxymandelonitrile dibenzoate can be isolated in substantially the same yield. The latter procedure eliminates isolating the intermediate hydroxybenzaldehyde benzoates.

The *p*-hydroxymandelonitrile dibenzoate is a white crystalline compound. When recrystallized from acetone, the crystals are small platelets melting from 144.5–145.5°. It was obtained in yields up to 67%.

Analysis: Theor. for $C_{22}H_{15}O_4N$: 3.92% N.
Found: 4.09% N.

The *o*-hydroxymandelonitrile dibenzoate was obtained in yields up to 45%. It is best recrystallized from *n*-propyl alcohol, giving pale yellow needle crystals melting from 92.0–92.5°.

[1] This melting point does not agree with that reported by Kopp, *Ann. Chem.*, 277 (1893) 350, who gives the melting point of *p*-hydroxybenzaldehyde benzoate as 72°. His method was repeated; a melting point and mixed melting point checked with that obtained above. Obviously there is some error as the melting point for the benzyl ether of *p*-hydroxybenzaldehyde is given as 72° by Worner, *Ber.*, 29, 142.

Analysis: Theor. for $C_{22}H_{15}O_4N$: 3.92% N.
Found: 4.13% N.

The *m*-hydroxymandelonitrile dibenzoate is a white crystalline compound. It was obtained after some difficulty by recrystallization as fine needles from toluene, cooled in a dry ice-alcohol bath. Its melting point is 118.5–119.5°.

Analysis: Theor. for $C_{22}H_{15}O_4N$: 3.92% N.
Found: 3.98% N.

SUMMARY

The synthesis of the dibenzoates of the three isomeric hydroxymandelonitriles has been investigated. They may be obtained:

(1) by treating the benzoate of the hydroxybenzaldehyde with an equivalent of alkali cyanide and benzoyl chloride;

(2) by treating the hydroxybenzaldehyde with an equivalent of pyridine, an equivalent of alkali cyanide and two equivalents of benzoyl chloride.

Of the three isomeric dibenzoates, the *o*- and the *m*- compounds have been, so far as known, prepared for the first time. The benzoate of *m*-hydroxybenzaldehyde is also reported for the first time.

The reduction behavior of these hydroxymandelonitrile dibenzoates will be investigated.

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(3) Francis and Davis, *J. Chem. Soc.*, 95 (1909), 1403.
(4) Hartung, *J. Am. Chem., Soc.*, 50 (1928), 3370.
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Christian Eijkman (1858–1930), discoverer of the cause of beriberi, shared the Nobel Prize for medicine in 1929 with Sir Frederick Hopkins for extensive vitamin research. To his satisfaction, the antineuritic vitamin was isolated as a pure crystalline substance by his countrymen, Janses and Donath, in the laboratory in Batavia which had risen out of his own primitive research quarters.

The Analysis of Sodium Acetate

By R. M. Hitchens, G. W. Ashworth* and W. H. Demaree†*

One common method for the determination of acetic acid in acetates has consisted of distillation from a strongly acid solution and titration of the distillate. Hurd and Fiedler (1) have reported encountering considerable difficulty in the assay of sodium acetate by this process. They found it necessary to maintain the volume of liquid being distilled at 200 cc. and to carry out the distillation over a period of ten hours to prevent high results from carryover of phosphoric acid.

These observations seem surprising to us since we have long used this method as a routine assay method for sodium acetate, both alone and in the presence of sodium carbonate and sodium chloride. In view of the troubles which Hurd and Fiedler experienced we are describing below the apparatus, procedure and the results obtained with known amounts of sodium acetate and acetic acid.

EXPERIMENTAL

Apparatus.—The distillation apparatus employed is illustrated in Fig. 1. It consists of a 250-cc., round-bottom flask connected by means of a ground-glass joint to a spray column filled with small glass helices which in turn leads to a condenser. A small reservoir is fitted to the top of the column to allow addition of water to the flask. The receiver is a 250-cc. Volhard absorption flask. This simple, compact still allows rapid distillation with no carryover. It is widely applicable to a variety of quantitative distillations.

Procedure.—Sufficient sample to contain about 0.02 Gm. mol of sodium acetate is added to the flask, along with 50 cc. of water and 15 cc. of 85% phosphoric acid. Twenty cc. of water are added to the receiver to act as a seal. The solution is distilled to a volume of about 20 cc., the flame removed, 20 cc. of water run in through the reservoir and the solution again distilled to 20 cc. For safety a second 20-cc. portion of water is added and distilled as before. The distillate is titrated with carbonate-free 0.5*N* sodium hydroxide solution, using phenolphthalein or thymol blue as indicator. The entire distillation consumes only 20 minutes.

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