

**Procedure for Analytical Degradation of Dibenzoyl Disulfide to Benzoic Acid.**—Reflux 1 g. of dibenzoyl disulfide with 30 cc. of 40% w/v potassium hydroxide solution in a 100-cc. round-bottomed flask for three hours. Cool, transfer to a separatory funnel (rinsing out flask with water), acidify with concentrated hydrochloric acid, and allow to cool. Shake the benzoic acid into ether, using five portions (total of 120 cc.) and collect the ether extracts in another separatory funnel. Wash the ether with 25 cc. of approximately 0.1 *N* hydrochloric acid, and then wash this aqueous portion with 15 cc. of ether. Combine the ether extracts and filter through cotton into a beaker, washing the cotton with fresh ether. Evaporate the ether with the aid of a stream of air. (Evaporation may be hastened by placing the beaker in a water-bath at not over 40° until 30 cc. remains; then remove from water-bath and complete removal with air.) Treat the residue with a solution of 1.5 g. of sodium bicarbonate in 50 cc. of water. Warm the mixture and allow it to stand until all the benzoic acid has dissolved; the sulfur will be practically

insoluble. Filter the liquid through a coarse filter paper into a separatory funnel and wash the paper thoroughly with water. Acidify the sodium benzoate solution with 10% hydrochloric acid, allow it to cool, and shake out the benzoic acid with chloroform, using five portions (total of 100 cc.). Collect the chloroform extracts in another separatory funnel. Wash the chloroform with 20 cc. of approximately 0.1 *N* hydrochloric acid, and then wash this aqueous portion with 10 cc. of chloroform. Filter the combined chloroform extracts through cotton into a tared beaker and evaporate the chloroform with the aid of a stream of air, placing the beaker in a water-bath at a temperature of not over 40° as before. Dry the benzoic acid overnight in a vacuum desiccator over sulfuric acid and weigh.

### Summary

A new practical method for preparing medicinally pure dibenzoyl disulfide is described.

CINCINNATI, OHIO

RECEIVED APRIL 6, 1936

[CONTRIBUTION FROM THE BURROUGHS WELLCOME AND CO., U. S. A., EXPERIMENTAL RESEARCH LABORATORIES]

## Some *N*-Aryl Barbituric Acids

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In connection with a study of the hypnotic action of certain unsymmetrical alkylaryl ureas,<sup>1</sup> it was desirable to obtain a series of 1-aryl barbituric acids, having the aryl group the same both for the ureas and for the barbituric acids. Two series were therefore prepared, one having 5,5-diethyl groups and the other 5,5-ethyl-*n*-butyl groups, the *N*-aryl groups in both series being phenyl, *o*-, *m*- and *p*-tolyl, *o*-, *m*- and *p*-anisyl, *o*-, *m*- and *p*-phenetyl, and  $\alpha$ - and  $\beta$ -naphthyl.

Hjort and Dox<sup>2</sup> have previously described briefly four of the diethyl compounds, but they failed to crystallize the 1-phenyl-5,5-ethyl-*n*-butyl compound, and to obtain the 1-*p*-ethoxyphenyl-5,5-ethyl-*n*-butyl derivative. The complete series (24) is here described. The pharmacological examination will be published in another place.

### Experimental

The ethyl diethylmalonate used was purchased. Ethyl *n*-butylethylmalonate was prepared in good yield by butylating ethyl ethylmalonate, in the usual way by means of sodium ethylate and *n*-butyl iodide. The ester boiled sharply at 109° (1.4 mm.) or 125–126° (11 mm.). When prepared by ethylating ethyl *n*-butylmalonate, no sharp boiling point could be obtained.

The barbituric acids were all prepared by the usual reaction. 0.05 mole (10.8 g. and 12.2 g.) of the dialkylmalonate was added to 0.2 atom (4.6 g.) of sodium dissolved in the minimum amount of absolute alcohol; 0.05 mole of the requisite urea was added and the mixture refluxed for four to five hours. The solution was then cooled, diluted with water, made just acid to Congo red, and most of the alcohol removed, by a current of air, on the steam-bath. The residue was extracted with ether and the ether washed three times with saturated sodium bicarbonate solution, to remove hydrolysis products (very little of the barbituric acid was lost). The washed ether was then extracted with 80 cc. of 10% sodium hydroxide solution, water being added if necessary. On acidification of the alkaline solution the barbituric acid separated, usually as an oil, and was extracted with ether or filtered off and recrystallized until pure, alcohol or slightly aqueous alcohol being the solvent, unless otherwise noted. A few of the ethyl-*n*-butyl compounds were very difficult to purify and required elaborate treatment.

The bicarbonate washings contain uncyclized compounds, which are usually small in amount. They can be isolated by acidifying the solution. Some were examined but are not recorded here. The residual ether contains unchanged urea, ester and decomposition products.

The barbituric acids are tabulated below. Variations in the foregoing procedure are indicated in footnotes. The compounds are all white, crystalline and tasteless. They are soluble in cold 5% sodium hydroxide solution, practically insoluble in water, slightly soluble to insoluble in petroleum ether (the low-melting ones are more soluble), moderately to readily soluble in ether, readily soluble to very soluble in alcohol, and from moderately soluble to

(1) Hjort, deBeer, Buck and Ide, *J. Pharmacol.*, **55**, 152 (1935).

(2) Hjort and Dox, *ibid.*, **35**, 155 (1929).

TABLE I  
 1-ARYL-5,5-DIETHYL BARBITURIC ACIDS

Aryl radical	Appearance	M. p., °C.	Formula	Analyses, % N	
				Calcd.	Found
Phenyl <sup>a</sup>	Small thick glittering plates	178	C <sub>14</sub> H <sub>16</sub> O <sub>2</sub> N <sub>2</sub>	10.76	10.83
<i>o</i> -Tolyl	Small glittering rect. plates	182	C <sub>15</sub> H <sub>18</sub> O <sub>2</sub> N <sub>2</sub>	10.22	10.13
<i>m</i> -Tolyl	Small striated prisms	133	C <sub>15</sub> H <sub>18</sub> O <sub>2</sub> N <sub>2</sub>	10.22	10.09
<i>p</i> -Tolyl <sup>b</sup>	Glittering prisms	155.5	C <sub>15</sub> H <sub>18</sub> O <sub>2</sub> N <sub>2</sub>	10.22	10.28
<i>o</i> -Anisyl	Nodules of small rect. plates	176.5	C <sub>15</sub> H <sub>16</sub> O <sub>4</sub> N <sub>2</sub>	9.65	9.66
<i>m</i> -Anisyl	Masses of fragmentary prisms	115-116	C <sub>15</sub> H <sub>16</sub> O <sub>4</sub> N <sub>2</sub>	9.65	9.73
<i>p</i> -Anisyl <sup>c</sup>	Thin flat prisms	129	C <sub>15</sub> H <sub>16</sub> O <sub>4</sub> N <sub>2</sub>	9.65	9.66
<i>o</i> -Phenetyl	Small flat rect. prisms	159	C <sub>16</sub> H <sub>20</sub> O <sub>4</sub> N <sub>2</sub>	9.21	9.18
<i>m</i> -Phenetyl	Small nodules of prisms	114	C <sub>16</sub> H <sub>20</sub> O <sub>4</sub> N <sub>2</sub>	9.21	9.08
<i>p</i> -Phenetyl <sup>d</sup>	Glittering small octahedra	160	C <sub>16</sub> H <sub>20</sub> O <sub>4</sub> N <sub>2</sub>	9.21	9.24
$\alpha$ -Naphthyl	Aggregates of small flat prisms	207	C <sub>17</sub> H <sub>18</sub> O <sub>2</sub> N <sub>2</sub>	9.03	9.01
$\beta$ -Naphthyl	Nodules of tiny prisms	146	C <sub>18</sub> H <sub>18</sub> O <sub>2</sub> N <sub>2</sub>	9.03	8.97

<sup>a</sup> Hjort and Dox<sup>2</sup> give m. p. 177°. <sup>b</sup> M. p. given as 152-153°. <sup>c</sup> M. p. given as 126-127°. <sup>d</sup> M. p. given as 152-153°.

 TABLE II  
 1-ARYL-5,5-ETHYL-*n*-BUTYL BARBITURIC ACIDS

Aryl radical	Appearance	M. p., °C.	Formula	Analyses, % N	
				Calcd.	Found
Phenyl <sup>a,b</sup>	Powder of minute nodules	ca. 70	C <sub>16</sub> H <sub>20</sub> O <sub>2</sub> N <sub>2</sub>	9.72	9.83
<i>o</i> -Tolyl <sup>c</sup>	Small stout rhombs	135	C <sub>17</sub> H <sub>22</sub> O <sub>2</sub> N <sub>2</sub>	9.27	9.33
<i>m</i> -Tolyl <sup>d,e</sup>	Powder of minute nodules	ca. 89	C <sub>17</sub> H <sub>22</sub> O <sub>2</sub> N <sub>2</sub>	9.27	9.28
<i>p</i> -Tolyl <sup>c</sup>	Small stout rounded prisms	142	C <sub>17</sub> H <sub>22</sub> O <sub>2</sub> N <sub>2</sub>	9.27	9.33
<i>o</i> -Anisyl <sup>c</sup>	Slightly glittering small prism. nodules	139.5	C <sub>17</sub> H <sub>22</sub> O <sub>4</sub> N <sub>2</sub>	8.80	8.82
<i>m</i> -Anisyl <sup>f</sup>	Chalky small nodules	102.5	C <sub>17</sub> H <sub>22</sub> O <sub>4</sub> N <sub>2</sub>	8.80	8.82
<i>p</i> -Anisyl	Bulky obscure prism aggregates	124	C <sub>17</sub> H <sub>22</sub> O <sub>4</sub> N <sub>2</sub>	8.80	8.84
<i>o</i> -Phenetyl	Granular tiny prisms	131	C <sub>18</sub> H <sub>24</sub> O <sub>4</sub> N <sub>2</sub>	8.43	8.48
<i>m</i> -Phenetyl <sup>a,b</sup>	Chalky bulky tiny nodules	84-85	C <sub>18</sub> H <sub>24</sub> O <sub>4</sub> N <sub>2</sub>	8.43	8.36
<i>p</i> -Phenetyl <sup>h</sup>	Granular small stout prisms	100	C <sub>18</sub> H <sub>24</sub> O <sub>4</sub> N <sub>2</sub>	8.43	8.39
$\alpha$ -Naphthyl <sup>i</sup>	Glittering stout prisms	182	C <sub>20</sub> H <sub>22</sub> O <sub>2</sub> N <sub>2</sub>	8.28	8.42
$\beta$ -Naphthyl	Dull white crusts	126	C <sub>20</sub> H <sub>22</sub> O <sub>2</sub> N <sub>2</sub>	8.28	8.32

<sup>a</sup> Not obtained crystalline by Hjort and Dox.<sup>2</sup> Reaction product was distilled (b. p. 203° at 0.5 mm.), rejecting the white sublimate first over. The distillate (stiff resin) was repeatedly recrystallized from carbon disulfide-heptane at 0°, a very tedious operation. The melting point is unsharp. The compound is very soluble in the usual solvents.

<sup>b</sup> These three compounds have recently been obtained crystalline, without the use of distillation, but distillation is preferable, and is essential in order to obtain seeding crystals.

<sup>c</sup> Repeatedly recrystallized from ether.

<sup>d</sup> As footnote <sup>a</sup>. B. p. 212° (0.6 mm.).

<sup>e</sup> After three recrystallizations from aq. alcohol, the product was partly dissolved in ether and the residue recrystallized from ether.

<sup>f</sup> Benzene used for extractions in place of ether.

<sup>g</sup> The reaction product was distilled (b. p. 225-230° at 1.2 mm.) rejecting the white sublimate first over. The distillate was dissolved in carbon disulfide, precipitated with petroleum ether, and kept at the b. p. (216° at 0.5 mm.) until about one-third had distilled over. The residue was crystallized from carbon disulfide-heptane and recrystallized from heptane, then ether, all at 0°. Many months were needed to obtain crystals.

<sup>h</sup> Hjort and Dox<sup>2</sup> obtained only an uncyclized product.

<sup>i</sup> After three recrystallizations from alcohol, the product was again extracted with ether and the ether extracted with another portion of sodium hydroxide solution. After acidification, the precipitate was recrystallized from alcohol, partly dissolved in ether, and the residue recrystallized from alcohol.

very soluble in benzene. The average yield, after drastic purification, is about one-half the weight of ester used, but considerable additional amounts may be recovered from the crystallization liquors. In general, the *m*-aryl compounds show greatly increased solubility and lower melting points, than the ortho and para isomers. The ethyl-*n*-butyl homologs melt lower than the corresponding diethyl compounds.

The ureas used are mostly described, with appropriate

references, by Buck, Hjort and deBeer.<sup>3</sup> *p*-Phenetyl urea was purchased from the Eastman Kodak Co.  $\alpha$ -Naphthyl urea<sup>4</sup> and  $\beta$ -naphthyl urea<sup>4a</sup> were made by the nitrourea-alcohol method.<sup>5</sup>

(3) Buck, Hjort and deBeer, *J. Pharmacol.*, **54**, 188 (1935).

(4) Beilsteins "Handbuch der organ. Chem.," 4 Aufl., XII, p. 1238; (4a) *ibid.*, p. 1292.

(5) Buck and Ferry, *THIS JOURNAL*, **58**, 854 (1936).

The analyses (micro-Dumas) were carried out by Mr. W. S. Ide. The melting points are corrected.

### Summary

A series of 1-aryl-5,5-diethyl barbituric acids, and a series of 1-aryl-5,5-ethyl-*n*-butyl barbituric acids, have been prepared, the substituent N-aryl

groups in both series being phenyl, *o*-, *m*- and *p*-tolyl, *o*-, *m*- and *p*-anisyl, *o*-, *m*- and *p*-phenetyl, and  $\alpha$ - and  $\beta$ -naphthyl. The aryl radicals were selected in order that comparisons might be made, pharmacologically, with a series of alkylaryl ureas having these radicals.

TUCKAHOE, NEW YORK

RECEIVED APRIL 23, 1936

[261ST CONTRIBUTION FROM THE INDUSTRIAL FARM PRODUCTS RESEARCH DIVISION, BUREAU OF CHEMISTRY AND SOILS, UNITED STATES DEPARTMENT OF AGRICULTURE]

## Biochemical Studies in the Genus *Rhizopus*. I. The Production of Dextro-Lactic Acid<sup>1</sup>

BY G. E. WARD, L. B. LOCKWOOD, O. E. MAY AND H. T. HERRICK

### Introduction

The production of lactic acid by microorganisms has, in general, been regarded heretofore as an attribute of bacteria, and not characteristic of the fungi. The prevailing conceptions regarding the relation of lactic acid to microbiological metabolism were summarized by Raistrick<sup>2</sup> in 1932: "It is a striking fact that lactic acid has never been reported as a mould metabolic product, although it is produced in larger or smaller quantities by many bacteria." Raistrick and other authors who have made similar statements have probably had in mind the biochemical activities of organisms of genera such as *Aspergillus*, *Penicillium* and *Fusarium*, which, indeed, have never been known to yield lactic acid. However, several investigators have reported lactic acid production in varying quantities by certain organisms of the genera *Rhizopus* and *Mucor*.

Since an accurate summary of the literature has never before appeared, and since some of the work is not conveniently reached or translated, it is briefly summarized here. In 1894, Eijkmann<sup>3</sup> suggested that the small quantity of acid produced by *Mucor rouxii* (*rouxianus*) was probably lactic acid, and Chrzaszcz<sup>4</sup> later confirmed this view. Shortly thereafter there were issued patents<sup>5</sup> which described the production of lactic acid by a mold termed "Lactomyces." The culture was of doubtful authenticity, but was probably a *Rhizo-*

*pus*. In 1911, Saito<sup>6</sup> reported that *Rhizopus chinensis* produced *l*-lactic acid when cultivated on glucose solutions, and in 1919, Ehrlich<sup>7</sup> stated that in cultures of certain *Rhizopus* species which produced principally fumaric acid, there occurred small quantities of *d*-lactic acid, succinic acid, and *l*-malic acid.

Takahashi and co-workers<sup>8</sup> showed that up to 38% of the fermented glucose was converted to *l*-lactic acid by certain species of *Rhizopus*. Varying quantities of fumaric acid, succinic acid, *l*-malic acid, formic acid, acetic acid and ethyl alcohol were also found in these cultures. In 1933, Takahashi and Asai<sup>9</sup> found that four species of *Mucor* produced traces of lactic acid, in addition to acetaldehyde, ethyl alcohol, pyruvic acid and succinic acid. Ethyl alcohol was the principal product of these fermentations.

In 1930, Kenji Miyaji<sup>10</sup> reported that a new *Monilia* species isolated from commercial cultures of soy sauce produced *d*-lactic acid and succinic acid when cultured on artificial media containing glucose.

The highest yields of *d*-lactic acid from carbohydrates by fungi heretofore obtained were recently reported by Kanel,<sup>11</sup> who found that a fungus resembling *Rhizopus japonicus* converted 38 to 40% of the consumed invert sugar or starch to this acid. Fumaric acid accumulated in older

(6) Saito, *Zentr. Bakt. Parasitenk. II*, **29**, 289 (1911).

(7) Ehrlich, *Ber.*, **52**, 63 (1919).

(8) Takahashi and Sakaguchi, *J. Agr. Chem. Soc. (Japan)*, **1**, 46 (1925); Takahashi, Sakaguchi and Asai, *Bull. Agr. Chem. Soc. (Japan)*, **2**, No. 5, 61 (1926).

(9) Takahashi and Asai, *Zentr. Bakt. Parasitenk. II*, **89**, 81 (1933).

(10) Kenji Miyaji, *Gifu Imp. Coll. of Agr. (Japan) Research Bull.*, **10** (1930).

(11) Kanel, *Microbiology (U. S. S. R.)*, **3**, 259 (1934).

(1) Presented in part before the Section of Biological Chemistry of the American Chemical Society, April, 1935, New York City, N. Y.

(2) Raistrick, *Ergebnisse Enzymforsch.*, **1**, 362 (1932).

(3) Eijkmann, *Zentr. Bakt. Parasitenk.*, **16**, 97 (1894).

(4) Chrzaszcz, *ibid.*, **II**, **7**, 326 (1901).

(5) Boullanger, British Patent 13,439 (1899); German Patent 118,063 (1901).