Podophyllic Acid "Trihydrate"[1]

WALTER J. GENSLER AND SHIH YI WANG

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Hydrolysis of picropodophyllin opens the lactone ring and gives podophyllic acid.[2] The acid, crystallized from a two-phase mixture of chloroform and water, was reported as a trihydrate.[3] Closer examination of this material has now shown that it is not a hydrate but is instead a nonstoichiometric combination with chloroform.

The effect of chloroform in inducing crystallization of podophyllic acid is striking. In the absence of chloroform, crystallization of podophyllic acid from water, if possible at all, is difficult, and usually gels are obtained. However, addition of chloroform promptly and smoothly precipitates the crystalline chloroform complex. Drying this material at 110° gives solvent free podophyllic acid, presumably the same as that reported before.[3]

EXPERIMENTAL[4]

Podophyllic acid "trihydrate," prepared essentially according to Borsche and Niemann,[3,5] showed melting points dependent on the rate of heating. Material recrystallized three times from water and chloroform melted at 153.5–154.5° (dec.) when the temperature was raised about 5° per minute. A slower rate of heating (*ca.* 1°/min.) tended to cause shrinking around 150° and fusion to a glass at 190–196° (dec.). When the temperature was raised very slowly, no change was apparent at the 154° region, and the solid melted at 203°. Loss of chloroform of solvation as well as relactonization could be responsible for this melting point behavior.

Anal. Calc'd for $C_{22}H_{24}O_9 \cdot 0.329(CHCl_3)$: C, 56.85; H, 5.20; Cl, 7.42; $CHCl_3$, 8.33. Found: C, 56.39; H, 5.02; Cl, 7.12; wt. loss on heating *in vacuo* overnight at 110°: 7.51.

Solution of the dried material in aqueous bicarbonate showed that lactonization did not occur. The melting point taken with a temperature increase of 1°/min. was 203–208° (dec.).

Anal. of the dried material. Calc'd for $C_{22}H_{24}O_9$: C, 61.10; H, 5.59. Found: C, 61.2; H, 5.6.

Other experiments furnished additional analytical data.

(A) Calc'd for chloroform complex, $C_{22}H_{24}O_9 \cdot 0.11(CHCl_3)$: C, 59.59; H, 5.45; Cl, 2.64; $CHCl_3$, 2.96. Found: 59.60; H, 5.42; Cl, 2.94; wt. loss on drying to constant weight at 110°, 2.84, 3.21. *Anal.* for the dried material: Found: C, 61.43; H, 5.56; Cl, 0.0.

(B) Calc'd for chloroform complex, $C_{22}H_{24}O_9 \cdot 0.176(CHCl_3)$: C, 58.74; H, 5.38; Cl, 4.13; $CHCl_3$, 4.64. Found: C, 59.19; H, 4.91; Cl, 4.13; wt. loss on drying at 110°, 4.72, 4.84. *Anal.* for the dried material: Found: C, 61.83; H, 5.68; Cl, 0.0.

The chlorine-free material as a mull in mineral oil showed an infrared absorption band at 5.91 μ. The specific rotation in absolute alcohol (0.864 g. per 100 ml.) was $(\alpha)_D^{25}$ −96.0.

Approximately 0.1 g. of chlorine-free podophyllic acid was dissolved in 15 ml. of almost boiling water. Filtration removed a small amount of solid. A very small amount of solid developed on standing at room temperature, and more appeared during refrigeration for three days. The solids formed in the cold mixture melted at 212–214° (dec.) and were insoluble in bicarbonate (picropodophyllin?). Addition of chloroform to the clear filtrate at room temperature resulted in immediate precipitate formation.

CHEMISTRY DEPARTMENT
BOSTON UNIVERSITY
BOSTON 15, MASS.

(1) This work was supported by grants-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) Kelly and Hartwell, *J. Nat. Cancer Inst.*, **14**, 967, 989 (1954).

(3) Borsche and Niemann, *Ann.*, **494**, 126 (1932).

(4) Analyses were performed by Dr. S. M. Nagy at Massachusetts Institute of Technology Microchemical Laboratory, and by Dr. C. K. Fitz, 115 Lexington Avenue, Needham Heights 94, Massachusetts.

(5) *Cf.* Hartwell and Schrecker, *J. Am. Chem. Soc.*, **73**, 2909 (1951).

Some Indole Derivatives Tested for Antitubercular Activity

ROBERT K. BROWN, ROBERT F. SNIDER, AND MARGARET D. STEVENSON

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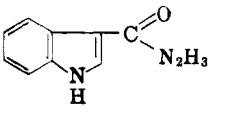
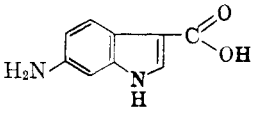
During the course of synthesis of indole derivatives in this laboratory interest in the compounds 3-indolecarboxylic acid hydrazide (I), 6-amino-3-indolecarboxylic acid (II), and 6-amino-3-indolecarboxylic acid hydrazide (III) arose because of the similarities of their structures to those of the known antitubercular compounds *p*-aminosalicylic acid (IV),[1] and isonicotinic acid hydrazide (V).[2]



I



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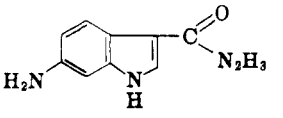
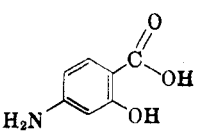
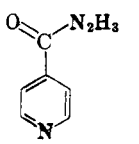


III



IV



V

Indole derivatives previously reported in connection with antitubercular activity are 3-indoleacetic acid hydrazide[3] and 3-indolecarboxaldehyde thiosemicarbozone.[4] The latter compound possesses high *in vitro* and *in vivo* activity against tubercle bacilli.

(1) Lehmann, *Lancet*, **250**, 15 (1946).

(2) Grunberg, Schnitzer, Leiwant, D'Ascensio, and Titsworth, *Quart. Bull. Sea View Hosp.*, **13**, 3 (1952).

(3) Yale, Losee, Martins, Perry, and Bernstein, *J. Am. Chem. Soc.*, **75**, 1933 (1953).

(4) Weller, Sell, and Gottshall, *J. Am. Chem. Soc.*, **76**, 1959 (1954).

Herein is reported the preparation of compounds I–III and the results of tests for antitubercular activity.

Acknowledgment. We are grateful to the National Cancer Institute of Canada for generous financial support. We wish to thank Dr. H. H. Fox of Hoffmann-La Roche, Inc. for carrying out the physiological tests.

EXPERIMENTAL

(All melting points are corrected.)

Indole-3-carboxylic acid hydrazide. A solution of 5 g. of ethyl indole-3-carboxylate[5] in a mixture of 25 ml. of methyl alcohol and 30 ml. of 64% hydrazine hydrate was kept at 45° for 6 hours. The volume of liquid was reduced to one half and then was diluted with water and cooled. The precipitate weighed 3.9 g. and melted at 225–233°. One crystallization from diluted ethyl alcohol gave 3.65 g. (75%) of the colorless hydrazide, m.p. 232–234°.

Anal. Calc'd for $C_9H_9N_3O$: C, 61.8; H, 5.2. Found: C, 61.8; H, 5.2.

Ethyl 6-aminoindole-3-carboxylate. A solution of 3 g. of ethyl 6-nitroindole-3-carboxylate[5] in 30 ml. of methyl alcohol containing 1 g. of Raney nickel[6] was treated with an excess of 64% hydrazine hydrate. Small quantities of the hydrazine was added at intervals so that the exothermic reaction maintained the temperature of the solution at 40–50°. When evolution of nitrogen became sluggish more hydrazine was added and the mixture was heated on the water-bath to 50° till the yellow color of the nitro compound disappeared. The nickel was removed, the alcohol boiled off under a vacuum, and the residual solid taken up in a small amount of 95% ethyl alcohol and decolorized. The heated filtrate was diluted with water and allowed to come to room temperature and then was cooled in a refrigerator. The colorless, needle-like crystals (1.78 g., 68%) melted at 151–152°. Recrystallization failed to raise the melting point. Majema and Kotake[5] obtained the same compound (m.p. 149–150°) by reduction of the ethyl 6-nitroindole-3-carboxylate with tin and hydrochloric acid.

Anal. Calc'd for $C_{11}H_{12}N_2O_2$: C, 64.7; H, 5.9. Found: C, 64.7; H, 6.1.

The same compound was obtained by reducing ethyl 6-nitroindole-3-carboxylate in methyl alcohol with Raney nickel and hydrogen (27 p.s.i.), yield 69%.

Colorless crystals of ethyl 6-acetaminoindole-3-carboxylate were obtained by treating a benzene solution of the amine with acetic anhydride. The precipitated compound crystallized from diluted alcohol melted at 269–270°.

Anal. Calc'd for $C_{13}H_{14}N_2O_3$: C, 63.4; H, 5.7. Found: C, 63.2; H, 5.6.

The *hydrochloride* of ethyl 6-aminoindole-3-carboxylate did not melt under 300°.

Anal. Calc'd for $C_{11}H_{13}ClN_2O_2$: C, 54.9; H, 5.5. Found: C, 55.0; H, 5.8.

6-Aminoindole-3-carboxylic acid hydrazide. A mixture of ethyl 6-aminoindole-3-carboxylate (1.12 g.) and excess 84% hydrazine hydrate was heated on the water-bath for one hour. The solution obtained was diluted with water (1:1) and when cooled deposited 0.8 g. of solid melting at 215–217°. Recrystallization from water or from aqueous ethyl alcohol raised the melting point to 224–225°.

Anal. Calc'd for $C_9H_{10}N_4O$: C, 56.8; H, 5.3. Found: C, 57.0; H, 5.1.

6-Aminoindole-3-carboxylic acid. A solution of 6 g. of 6-nitroindole-3-carboxylic acid in 200 ml. of methyl alcohol was shaken with freshly prepared Raney nickel and hydrogen (35 p.s.i.) until the yellow color disappeared. The nickel was extracted several times with hot methyl alcohol and the combined alcohol solutions were decolorized and reduced under a vacuum to a small volume. The alcohol solution, diluted with water, was cooled over-night and afforded 3.6 g. (74%) of the amino acid, m.p. 202–204° dec.: Crystallization from diluted methyl alcohol gave a melting point of 207–209° dec.

Attempts at crystallization from water resulted in decarboxylation to the 6-aminoindole.

A quantity of 6-aminoindole-3-carboxylic acid (0.4 g.) was dissolved in 15 ml. of 2 *N* sodium hydroxide and the solution was shaken with excess benzoyl chloride. The mixture was neutralized with hydrochloric acid and the precipitate (0.4 g.) when crystallized from 98% ethyl alcohol gave 6-benzoylaminoindole-3-carboxylic acid, m.p. 260–262°.

Anal. Calc'd for $C_{16}H_{12}N_2O_3$: C, 68.6; H, 4.3. Found: C, 68.5; H, 4.3.

A large excess of benzoyl chloride afforded only the monobenzoyl derivative described above.

Attempts to form the N-benzoyl-6-nitroindole-3-carboxylic acid were unsuccessful.

6-Nitroindole-3-carboxylic acid hydrazide. One gram of ethyl 6-nitroindole-3-carboxylate in 15 ml. of 95% hydrazine hydrate was warmed to 65° whereupon solution occurred. The heating was continued at 50° for 6 hours. The cold liquid deposited 0.9 g. of solid which did not melt below 300°. Attempts at crystallization from common solvents were unsuccessful.

Anal. Calc'd for $C_9H_8N_4O_3$: C, 49.5; H, 3.7. Found: C, 49.7; H, 3.4.

The nitrohydrazide was suspended in methyl alcohol (60 ml.) and reduced with Raney nickel and hydrazine until the yellow color as well as the suspension disappeared. Removal of the nickel, decolorization, and concentration of the liquid to 20 ml. gave a precipitate whose melting point and mixture melting point showed it to be 6-aminoindole-3-carboxylic acid hydrazide.

Animal tests. White mice, injected intravenously with *M. tuberculosis* H37 Rv were treated with a tolerated dose of the compound to be tested incorporated in the diet. None of the compounds I–III showed evidence of anti-tuberculous activity.

(5) Majima and Kotake, *Ber.*, **63**, 2237 (1930).

(6) Covert and Adkins, *J. Am. Chem. Soc.*, **54**, 4116 (1932).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF ALBERTA
EDMONTON, ALBERTA, CANADA