at room temperature before removing (at 30–40°) the solvent *in vacuo*. Ammonium chloride solution was added to the residue and the crude waxy product collected, washed with water, and recrystallized from acetone; yield, 1.9 g. (38%), m.p. 110–120°. Two recrystallizations from acetone-water, followed by one from ether, gave an analytical sample as colorless crystals, m.p. 138.5–139.5°.

Anal. Calcd. for $C_{21}H_{27}NO_6$: C, 64.76; H, 6.99; N, 3.60. Found: C, 64.85; H, 6.94; N, 3.79.

α-[2-(Diethylamino)ethyl]-α-(3,4,5-trimethoxyphenyl)piperonyl alcohol (IVb). A sample of this substance was prepared employing the general procedure described above (*cf.*, IVa). The crude product from a solution of 3,4-methylenedioxybromobenzene (3.6 g., 0.018 mole) in anhydrous tetrahydrofuran (30 ml.), ethereal 2*N* butyllithium (9 ml.), and a solution of β-*N*-diethylamino-3,4,5-trimethoxypropiophenone (IIIb, 5.3 g., 0.018 mole), prepared from the hydrochloride derivative[13] in 30 ml. of anhydrous tetrahydrofuran, was recrystallized from ethanol-water; weight 3.5 g. (46.7%), m.p. 77–80°. Four recrystallizations from methanol gave a pure sample of colorless crystals melting at 108°.

Anal. Calcd. for $C_{23}H_{31}NO_6$: C, 66.16; H, 7.49; N, 3.36. Found: C, 66.02; H, 7.60; N, 3.54.

α-[2-(N-Piperidino)ethyl]-α-(3,4,5-trimethoxyphenyl) piperonyl alcohol (IVe). To a solution of 3,4-methylenedioxy bromobenzene (4.02 g., 0.02 mole) in 20 ml. of anhydrous tetrahydrofuran was added 10 ml. of 2*N* ethereal butyllithium followed by β-*N*-piperidino-3,4,5-trimethoxypropiophenone (IIIe, 6.1 g., 0.02 mole) dissolved in anhydrous tetrahydrofuran (100 ml.) as described for the preparation of IVa. Recrystallization of the crude product, 7.1 g. (79%), m.p. 80–100°, from ethanol gave a colorless crystalline analytical sample melting at 146°.

Anal. Calcd. for $C_{24}H_{31}NO_6$: C, 67.11; H, 7.28; N, 3.26. Found: C, 67.41; H, 7.42; N, 3.45.

α-[2-(N-Morpholino)ethyl]-α-(3,4,5-trimethoxyphenyl)-piperonyl alcohol (IVd). This compound was obtained by the procedure outlined for IVa, employing 3,4-methylenedioxybromobenzene (2.21 g., 0.011 mole) in anhydrous tetrahydrofuran (15 ml.), 5.5 ml. of 2*N* ethereal butyllithium and 3.43 g. (0.011 mole) of β-*N*-morpholino-3,4,5-trimethoxypropiophenone (IIIf) dissolved in 140 ml. of tetrahydrofuran. The crude product weighed 3.6 g. (76%) and melted at 156–160°. The analytical sample recrystallized from ethanol-water as colorless crystals, m.p. 161–162°, $\gamma_{max}^{CHCl_3}$ 3300–2850 cm.$^{-1}$

Anal. Calcd. for $C_{23}H_{29}NO_7$: C, 64.02; H, 6.77; N, 3.25. Found: C, 64.48; H, 6.92; N, 3.44.

α-[2-(N-Morpholino)ethyl]-α-(p-methoxyphenyl) piperonyl alcohol (IVe). The crude product prepared as illustrated above (*e.g.*, IVa) from ethereal 2*N* butyllithium (20 ml.), 3,4-methylenedioxybromobenzene (8.04 g., 0.04 mole) in anhydrous tetrahydrofuran (30 ml.), and β-*N*-morpholino-4-methoxypropiophenone[16] (IIIh, 10 g., 0.04 mole) in 80 ml. of anhydrous tetrahydrofuran weighed 13.3 g. (89.5%), and melted at 110–135°. Repeated recrystallization from either ethanol, ethanol-water, or benzene–petroleum ether (b.p. 60–90°) yielded pure colorless crystals, m.p. 143–143.5°, $\gamma_{max}^{CHCl_3}$ 3300–2850 cm.$^{-1}$

Anal. Calcd. for $C_{21}H_{25}NO_5$: C, 67.90; H, 6.78; N, 3.77 Found: C, 68.18; H, 6.65; N, 3.92.

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[16] T. Okuda, *Yakugaku Zasshi*, **76**, 1 (1956). *Chem. Abstr.*, **50**, 13029 (1956).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MAINE]

Potential Cancerocidal Agents. II. Synthesis of 6,7-Methylenedioxycarbostyril[1]

GEORGE R. PETTIT AND MALDA V. KALNINS

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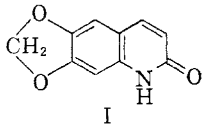
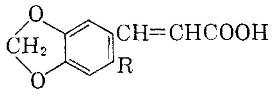
An unequivocal synthesis of 6,7-methylenedioxycarbostyril has been accomplished. Previous reports describing the preparation of this substance have been examined and reinterpreted.

During the course of a continuing search for a substance with useful antitumor activity, it was desirable to prepare 6,7-methylenedioxycarbostyril (I) for biological evaluation.[2]

Although the synthesis of 6,7-methylenedioxycarbostyril (I) had been reported by both Narang[3] and Borsche,[4] certain inconsistencies made questionable the result of each procedure. Reduction of 3,4-methylenedioxy-6-nitrocinnamic acid (IIa) with aqueous ammonia and ferrous sulfate, followed by acidification, had been claimed[3] to yield the carbostyril (I), m.p. 205°. The validity of this conclusion was doubtful in view of the experimental conditions employed and the earlier work of Perkin[5] in which the same reaction sequence had been reported to yield 3,4-methylenedioxy-6-aminocinnamic acid (IIb, brown needles, m.p. 205–207°). However, Narang[3] noted that his product apparently did not contain an amino or carboxylic acid group. Several years later, the room temperature reaction between acetic anhydride and the Schiff



I



IIa. R = NO_2
b. R = NH_2

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[1] This investigation was aided by Grant T-79 from the American Cancer Society.

[2] This work constitutes part of a study concerned with the synthesis of nitrogen compounds based on certain structural features of the tumor-damaging natural product, podophyllotoxin. Consult: G. R. Pettit, and D. S. Alkalay, *J. Org. Chem.*, **25**, 1363 (1960), for the preceding paper in this series.

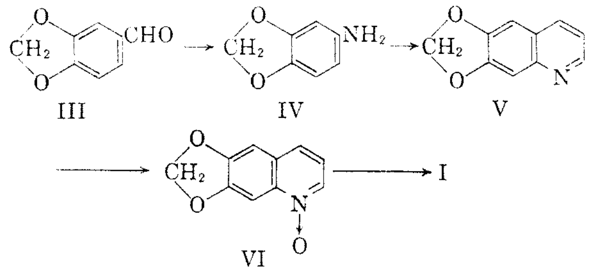
[3] K. S. Narang, J. N. Ray, and T. Das Sachdeva, *J. Indian Chem. Soc.*, **13**, 260 (1936).

[4] W. Borsche and W. Ried, *Ann.*, **554**, 269 (1943).

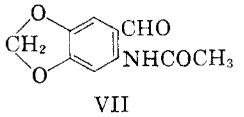
[5] F. M. Perkin, *J. Chem. Soc.*, **59**, 150 (1891).

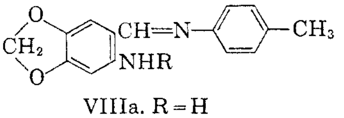
base VIIIa was found to yield a yellow compound melting at 158-159°, which was again assigned the 6,7-methylenedioxycarbostyril (I) structure.[4]

In our hands, reduction of 3,4-methylenedioxy-6-nitrocinnamic acid (IIa) yielded IIb as golden yellow platelets melting at 200-202° dec., readily soluble in dilute sodium bicarbonate. Treating the amino acid (IIb) with hot hydrochloric acid, followed by neutralization, gave a colorless crystalline product melting at 351-353° dec. The infrared spectrum and elemental analysis of the latter compound were clearly in agreement with the 6,7-methylenedioxycarbostyril (I) representation. An unequivocal synthesis of the carbostyril I, therefore, became desirable. The following reaction sequence (III → I) was selected for this purpose. Piperonal (III) was converted *via* its amino derivative (IV) to 6,7-methylenedioxyquinoline (V). Peracetic acid oxidation of V yielded 6,7-methylenedioxyquinoline *N*-oxide (VI) which smoothly rearranged under the influence of boiling acetic anhydride to 6,7-methylenedioxycarbostyril (I). The authentic sample of I, m.p. 351-353°, was identical with the product obtained from the amino acid IIb.

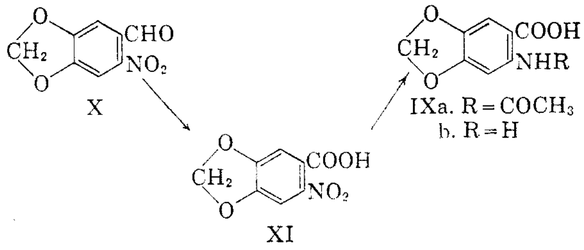


In a reinvestigation of the transformation which occurs when the amine VIIIa is placed in acetic anhydride,[4] it was found that two products could be isolated. Fractional recrystallization of the reaction product from methanol, gave a colorless crystalline compound, m.p. 161-162°, and a pale yellow crystalline substance melting at 186-188°. Elemental analyses and Rast molecular weight determinations suggested empirical formulas $C_{10}H_9NO_4$ and $C_{16}H_{16}N_2O_3$ respectively for these products. This information, combined with the results of an infrared spectral study, implied structures VII and VIIIb. This structural assignment was partially confirmed when it was shown that heating with *p*-toluidine converted the product melting at 161-162° (VII) to the higher melting (186-188°) substance (VIIIb). However, oxidation of the aldehyde VII with either silver oxide or potassium permanganate afforded an acid melting at 239-240°, while 6-acetaminopiperonylic acid (IXa) had been reported by Bogert and Elder[6] to melt at 124-125°. Consequently, an unambiguous synthesis of the acid IXa was undertaken in order to provide an authentic specimen for comparison purposes.


VII


VIIIa. R = H
b. R = COCH$_3$

Oxidation of 6-nitropiperonal (X) led to 6-nitropiperonylic acid (XI) which was reduced with ferrous sulfate, in hot aqueous ammonia solution, to the corresponding amino acid IXb. Treating IXb with warm acetic anhydride gave 6-acetaminopiperonylic acid, m.p. 240-241°. The latter sample (IXa) was identical with the product arising from oxidation of the compound melting at 161-162° (VII).



The room temperature reaction between the Schiffs' base VIIIa and acetic anhydride, therefore, yields the acetanilide derivatives VII and VIIIb. For this reason, it also appears likely that the reaction between the *p*-toluidine derivative of 3,4-dimethoxy-6-aminobenzaldehyde and acetic anhydride may, in fact, yield the corresponding acetanilide derivative and not 6,7-dimethoxycarbostyril as reported by Borsche and Ried.[4]

EXPERIMENTAL[7]

6,7-Methylenedioxycarbostyril (I). A. *From 3,4-methylenedioxy-6-aminocinnamic acid* (IIb). A stirred suspension of 3,4-methylenedioxy-6-nitrocinnamic acid[6,8] (IIa, 70 g.) in 1.2 l. of water containing 750 g. of ferrous sulfate heptahydrate was heated to 80° before adding 600 ml. of 28% ammonium hydroxide. The source of heat was removed and stirring was continued over a 45-min. period. The black colored reaction mixture was filtered through Celite and the filtrate was acidified to *p*H 4 with 280 ml. of hydrochloric acid. The orange-yellow colored crystalline product (IIb) weighed 37 g. (60%) and melted at 180-190° dec. Recrystallization from dimethylformamide-water gave golden-yellow platelets, m.p. 200-202° dec.

Anal. Calcd. for $C_{10}H_9NO_4$: C, 57.97; H, 4.38; N, 6.76. Found: C, 57.99; H, 4.41; N, 7.09.

A mixture of 3,4-methylenedioxy-6-aminocinnamic acid (IIb, 5 g.) and 75 ml. of 18% hydrochloric acid was heated at steam bath temperature for 2 hr. The resulting dark brown colored solid was collected and washed successively with dilute sodium bicarbonate solution and water. Recrystallization from acetic acid (Norit-A) afforded colorless

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(6) M. T. Bogert and F. R. Elder, *J. Am. Chem. Soc.*, **51**, 532 (1929).

(7) Melting points were observed using open Kimble glass capillaries and are uncorrected. Microanalyses were provided by Dr. A. Bernhardt, Max-Planck Institut, Mülheim, Germany. Infrared spectra were recorded by Mr. E. Thomas, Department of Chemistry, University of Maine.

(8) The general procedure described by J. F. Kefford, *J. Chem. Soc.*, 1209, (1940), provides a convenient method for the preparation of this compound.

crystals of 6,7-methylenedioxycarbostyril (I); yield, 2 g. (44%), m.p. 351–353° dec.

B. *From 6,7-methylenedioxyquinoline N-oxide* (VI). Eight milliliters of 30% hydrogen peroxide was added to a solution of 6,7-methylenedioxyquinoline (V, 7 g.)[9] in 50 ml. of glacial acetic acid. After warming the reaction mixture at 70° for 16 hr., it was diluted to 100 ml. with water. The crystalline *N*-oxide (VI) was collected following adjustment of the cool reaction mixture to *p*H 5–6 with aqueous ammonia. Recrystallization from ethanol (Norit-A) gave colorless needles of 6,7-methylenedioxyquinoline *N*-oxide monohydrate melting at 179–180°; yield 4.1 g. (54%).

Anal. Calcd. for $C_{10}H_9NO_4$: C, 57.97; H, 4.38; N, 6.76. Found: C, 58.21; H, 4.45; N, 7.22.

A solution of 6,7-methylenedioxyquinoline *N*-oxide (VI, 1.8 g.) in 15 ml. of acetic anhydride was heated at reflux for 5 hr. The reaction mixture was then concentrated *in vacuo*. The rust colored product which separated was collected by filtration and washed with several small portions of water; yield, 1.2 g. (63%), m.p. 350–353° dec. A pure sample (I) recrystallized from acetic acid (Norit-A)–water as colorless crystals melted at 352–353° dec., λ_{max}^{KBr} 5.95, 6.38, 6.69, 6.95, 7.97, 9.63, 10.4, 10.6 and 11.5 μ.

Anal. Calcd. for $C_{10}H_7NO_3$: C, 63.49; H, 3.73; N, 7.41. Found: C, 63.43; H, 3.81; N, 7.36.

Product I was found by mixture melting point and infrared spectral comparison to be identical with the sample of 6,7-methylenedioxycarbostyril prepared from 3,4-methylenedioxy-6-aminocinnamic acid (IIb).

Acetylation of 6-aminopiperonylidene-p-toluidine (VIIIa). A solution of 6-aminopiperonylidene-*p*-toluidine[4,6] (VIIIa, 2.5 g.) and 5 ml. of acetic anhydride (without prior purification) was allowed to remain at room temperature for 5 days. The orange colored crystalline product was collected and washed with hot methanol. The yellow colored crystalline residue weighed 1.2 g. and melted at 184–186°. Recrystallization from methanol afforded a pure sample of 6-acetaminopiperonylidene-*p*-toluidine (VIIIb) as yellow needles, m.p. 186–188°, λ_{max}^{KBr} 3.05 and 5.90 μ.

Anal. Calcd. for $C_{16}H_{16}N_2O_3$ (284): C, 67.59; H, 5.67; N, 9.85. Found: C, 67.61; H, 5.46; N, 9.65; mol. wt. (Rast), 279.

Cooling the combined methanol washings resulted in the isolation of 0.4 g. of *6-acetaminopiperonal* (VII). The colorless needles melted at 161–162° (lit.,[4] m.p. 161–162°) after recrystallization from methanol; λ_{max}^{KBr} 3.05, 5.88, 5.98, and 6.14 μ.

Anal. Calcd. for $C_{10}H_9NO_4$ (207): C, 57.97; H, 4.38; N, 6.76. Found: C, 57.60; H, 4.32; N, 6.83; mol. wt. (Rast), 198.

When the reaction was carried out employing redistilled acetic anhydride and a 15-min. reaction period, only the Schiff base VIIIb was obtained; while addition of water (1:2 water–acetic anhydride) and extension of the reaction time to 24 hr. led to almost exclusive production of 6-acetaminopiperonal (VII).

The presence of 6,7-methylenedioxycarbostyril (I) was not detected.

6-Acetaminopiperonylidene-p-toluidine (VIIIb). A mixture of *p*-toluidine (0.10 g.) and 0.20 g. of 6-acetaminopiperonal, isolated from the acetylation of VIIIa, was heated at 120° *in vacuo* (water-aspirator for *ca.* 20 min.). Recrystallization from methanol gave yellow needles (0.10 g.), m.p. 186–188°. Mixture melting point and infrared spectral comparison with the substance isolated above, (VIIIb, m.p. 186–188°), established the identical nature of both products.

6-Acetaminopiperonylic acid (IXa). A. *By oxidation of 6-acetaminopiperonal* (VII). A 1.8-g. sample of 6-acetaminopiperonal, prepared as described above (acetylation of VIIIa), 3.1 g. of silver nitrate, and 14 ml. of water was heated to reflux before slowly, and with frequent agitation, adding sodium hydroxide (1.2 g.) in 14 ml. of water. Heating at reflux was continued until the silver mirror dispersed. After the reaction mixture was filtered and the filtrate acidified with dilute hydrochloric acid, the light gray colored crystalline product was collected and recrystallized from methanol. The colorless crystals weighed 1.6 g. (82.5%), m.p. 239–240° dec. The product was shown (mixture melting point and infrared spectral comparison) to be identical with an authentic sample of 6-acetaminopiperonylic acid (IXa) prepared as described below (procedure B).

B. *From 6-aminopiperonylic acid* (IXb). The 6-nitropiperonylic acid[10] employed in this procedure was prepared by silver oxide oxidation of 6-nitropiperonal.[11]

A mixture of 6-nitropiperonylic acid (XI, 8.0 g.) ferrous sulfate (97 g.), water (150 ml.) and 1 ml. of concd. hydrochloric acid was heated to 90° before adding 80 ml. of aqueous ammonia (28%) over a 20-min. period. Heating on the steam bath and stirring were continued for 45 min. The hot reaction mixture was then filtered through Celite and after washing the insoluble material thoroughly with water, the combined filtrate was acidified to *p*H 4.8 with hydrochloric acid. At this point, it was found that the amino acid was still absorbed on the iron oxides. Consequently, the filter cake was suspended (with stirring) in 300 ml. of water and acidified to *p*H 2 with hydrochloric acid. The mixture was filtered and the filtrate adjusted to *p*H 4.8 with aqueous ammonia. The dark colored precipitate was collected, dried, and heated at steam bath temperature with acetic anhydride for 1.5 hr. After cooling, the grayish-brown crystals were collected and recrystallized from methanol (Darco) to give colorless crystals, yield 0.7 g., m.p. 240–241° dec., (*cf.* Ref. 6).

Anal. Calcd. for $C_{10}H_9NO_5$: C, 53.81; H, 4.06; N, 6.28. Found: C, 53.97: H, 4.14; N, 6.10.

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(9) A. Sonn and F. Benirschke, *Ber.*, **54**, 1730 (1921).

(10) J. Jobst and O. Nesse, *Ann.*, **199**, 70 (1879).

(11) J. B. Ekeley and M. S. Klemme, *J. Am. Chem. Soc.*, **50**, 2711 (1928).