The residue (4.7 g.) obtained on evaporation of the supernatant and washings from the osmate ester precipitate was chromatographed on alumina. Benzene eluted 3.9 g. of codeinone dimethyl ketal (total recovery, 4.3 g., 64%), and chloroform eluted a second fraction which was crystallized from benzene and the crystals (m.p. 155–165°) were rechromatographed. They were put on the column with benzene and developed with benzene and then chloroform. Evaporation of the chloroform and crystallization of the residue from benzene–hexane gave 310 mg. of **N-formylnorcodeinone dimethyl ketal**, m.p. 183–184°, $[\alpha]^{21}\text{D}$ −206° (*c* 1.0).

Anal. Calcd. for $C_{20}H_{25}O_5N$: C, 66.9; H, 7.0; O, 22.3. Found: C, 66.9; H, 6.6; O, 22.0.

N-Formylnorcodeinone dimethyl ketal had a strong absorption in the infrared at 5.98 μ, it absorbed one mole of hydrogen on hydrogenation with 5% palladized carbon in ethanol, and it showed no basic properties when titrated with perchloric acid in acetic acid. Formic acid was identified as the volatile acid after hydrolysis.[14]

Codeinone Dimethyl Ketal-N-oxide.—Codeinone dimethyl ketal was converted to its N-oxide using the monoperphthalic acid in acetone procedure.[2b] A 75% yield of material was obtained which on crystallization from benzene melted at 191–192°, $[\alpha]^{21}\text{D}$ −293° (*c* 0.84). On mixing with the N-formylnorcodeinone dimethyl ketal (m.p. 183–184°), the melting point was depressed 40°.

Anal. Calcd. for $C_{20}H_{25}O_5N$: C, 66.9; H, 7.0. Found: C, 66.6; H, 6.9.

Periodate Cleavage of Glycols.—In each case, the rate of consumption of periodate by the respective glycol was determined at *p*H 5 (acetate buffer) or *p*H 6 (phosphate buffer) and at 25°. The alkaloid concentration was approximately 10^{-3} *M*, the periodate was 4×10^{-3} *M* and the oxidation was followed by withdrawing aliquots and analyzing by standard procedures.[15]

Preparative experiments were similar to the rate determinations, except that the alkaloid concentration was increased two- to sixfold (as solubility permitted), and the periodate was present only in slight excess (*ca.* 20%) over that required for the desired extent of oxidation. After the oxidation had proceeded to the desired point (checked by aliquot analysis), ethylene glycol was added to consume excess periodate, the solution was made alkaline and the alkaloid was extracted with ether or chloroform.

A. **Cleavage of 7,8-Dihydroxydihydrodesoxycodeine** (I).—In less than 30 min., glycol I had consumed 100 mole % of periodate. A second mole was consumed after 2 hr., and oxidation continued at this slower rate beyond 300 mole %.

From 343 mg. of glycol I, treated with 120 mole % periodate for 1 hr., there was isolated 305 mg. of a brownish oil by adjustment of the *p*H to 8.5, extraction with chloroform and evaporation of the chloroform. This material resisted all attempts at crystallization. It gave a strong phenolic test with diazotized sulfanilic acid, exhibited infrared absorption at 2.74(m), 5.80(w) and 5.98(s) μ, and in the ultraviolet had $\lambda_{\max}^{\text{EtOH}}$ 236, ε 13600.

B. **Cleavage of 7,8-Dihydroxydihydrocodeine** (II).—Within 10 minutes, triol II had consumed 100 mole % of periodate and 14 hr. was necessary for the consumption of a second mole after which consumption ceased.

From larger scale oxidations, stopped after 100 mole % and 200 mole % consumption of periodate, respectively, no crystalline material or derivatives could be obtained. The alkaloidal material, recovered in over 80% yield, showed infrared absorption in the saturated and unsaturated carbonyl regions.

C. **Cleavage of 6-Methyl-7,8-dihydroxydihydrocodeine** (III).—Oxidations at *p*H 3, 5 and 6 all exhibited 100 mole % consumption of periodate within minutes, and there was very little if any further oxidation.

A 520-mg. sample of the triol III, oxidized by the general procedure above at *p*H 5, gave 390 mg. (75% yield) of the one-mole oxidation product, m.p. 182–183°. After crystallization from benzene–methylcyclohexane, it melted at 184–185°, $[\alpha]^{21}\text{D}$ −113° (*c* 1.0).

Anal. Calcd. for $C_{19}H_{23}O_5N$: C, 66.1; H, 6.7; O, 23.2. Found: C, 65.9; H, 6.5; O, 23.4.

With hydroxylamine, phenylhydrazine and 2,4-dinitrophenylhydrazine no derivatives were obtained. The Tollens, Schiff and Fehling tests were negative.

Crystallization from water gave material of m.p. 219–220°, $[\alpha]^{21}\text{D}$ −85° (*c* 0.9).

Anal. Calcd. for $C_{19}H_{23}O_5N$: C, 66.1; H, 6.7; O, 23.2. Found: C, 66.3; H, 6.7; O, 23.2.

D. **Cleavage of 7,8-Dihydroxydihydrocodeinone Dimethyl Ketal** (IV).—Consumption of 100 mole % of periodate by glycol IV required 1 hr. after which no further oxidation occurred.

Preparative oxidations were performed at *p*H 6 and the final extraction of alkaloidal material was made after adjusting the aqueous phase to *p*H 11 or 8.5. Yields of oxidation product in each case were over 80%. The material extracted at *p*H 11 was dissolved in methanol and precipitated with ether. After repeating this procedure, crystalline material, m.p. 228–230°, was obtained. This material could not be recrystallized from a variety of solvents and frequently was very difficult to obtain crystalline.

Anal. Calcd for $C_{20}H_{25}O_6N$ (3 OCH_3): OCH_3, 24.8. Found: OCH_3, 25.0.

Extraction at *p*H 8.5 afforded crude material which on crystallization from benzene–cyclohexane melted at 96–97°, $[\alpha]^{21}\text{D}$ −100 (*c* 0.6).

Anal. Calcd. for $C_{20}H_{25}O_6N$: C, 64.0; H, 6.7; O, 25.6. Found: C, 63.8; H, 6.9; O, 25.5.

Either product, dissolved in aqueous acid, and then extracted at the appropriate *p*H, gave the product characteristic of the *p*H of the extraction.

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(14) D. J. Bell, A. Palmer and A. T. Johns, *J. Chem. Soc.*, 1536 (1949).

(15) E. L. Jackson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 341.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY AND THE DEPARTMENT OF ENTOMOLOGY, UNIVERSITY OF WISCONSIN]

Isolation and Synthesis of an Insect Resistance Factor from Corn Plants

BY EDWARD E. SMISSMAN, JULES B. LAPIDUS AND STANLEY D. BECK

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The procedure for the isolation of one of three chemical factors which have been shown to be responsible for the resistance of corn plants to attack by the European corn borer is given. The compound was shown to be 6-methoxybenzoxazolinone. The procedure for a chemical synthesis of this resistance factor is reported.

In 1951, Beck[1] proposed the existence, in the corn plant, of a substance which inhibits the growth of the European corn borer. In subsequent work[2–4] it has been shown that the amount of

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(1) S. D. Beck, *Proc. North. Cent. Br. Amer. Assn. Econ. Ent.*, **6**, 58 (1951).

(2) S. D. Beck and J. D. Stauffer, *Ann. Ent. Soc. Amer.*, **50**, 166 (1957).

(3) R. S. Loomis, S. D. Beck and J. F. Stauffer, *Plant Physiol.*, in press.

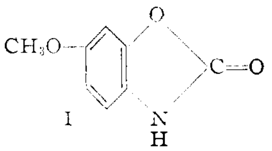
(4) S. D. Beck, *J. Insect Physiology*, in press.

material present in the plant is related to the age of the plant, the tissue from which it is extracted and to the strain of corn used as a source. The material which appeared to be responsible for the resistance of the young corn plant to attack by corn borer was designated as "Factor A" and was isolated from the dried and powdered corn plant by hot aqueous extraction. The aqueous solution was extracted with ether and the ether-soluble material was chromatographed.

Recently[4] evidence for two additional factors has been obtained. A discussion of their isolation and chemistry will be the subject of a future report. "Factor A" is a white crystalline substance, m.p. 154–155°, $\lambda_{\max}^{\mathrm{EtOH}}$ 230, 287 mμ. It has an empirical formula $C_8H_7NO_3$ and ebullioscopic methods indicated its molecular weight to be 165. The compound was shown to contain one methoxy group by micro Zeisel determination, and it was found to be soluble in 5% aqueous sodium hydroxide but insoluble in 5% aqueous sodium bicarbonate. The compound was optically inactive; it gave no reaction with 2,4-dinitrophenylhydrazine or with ferric chloride. However, the material formed a benzamide and a benzenesulfonamide with ease when amide formation was carried out in pyridine solution.

"Factor A" was stable to acid hydrolysis but hydrolyzed rapidly in alkali. On acidification and extraction a compound was isolated which was shown to be the hydrochloride of 2-amino-5-methoxyphenol.

In 1955, Virtanen, *et al.*,[5] showed that an antifusarium factor found in rye seedlings was benzoxazolinone. This finding coupled with our data would indicate "Factor A" to be 6-methoxybenzoxazolinone (I). While we were investigating this compound, Virtanen and co-workers[6] postulated this structure for a compound which they were able to isolate from wheat and maize. They gave no experimental data in this report. We reported the preparation of 6-methoxybenzoxazolinone and its identity with the natural product.[7a,b] Our method of preparation involved the nitration of 3-acetoxyanisole to give 2-nitro-5-methoxyphenol which was then reduced to the corresponding amino compound and fused with urea to yield 6-methoxybenzoxazolinone (I). Infrared spectra of the synthetic and naturally occurring products were identical. The spectra were obtained in chloroform solution and the following assignments were given to the major peaks.



I

Wave length, μ	Assignments
2.9	N-H stretching
3.2; 3.3	C-H stretching (benzene)
5.7	C=O (carbamate)
3.2; 6.3	C=C skeletal inplane vibr. (benzene)
7.6	C-N stretching (Ar-NH-R)

Experimental

Extraction and Isolation Procedure.—The shoots and leaves of a resistant strain field corn were used as starting material.

After drying and powdering, 100 g. of plant material was mixed with two liters of distilled water and boiled until the fibrous material settled. The hot mixture was filtered and the marc discarded. After the dark brown solution (*ca.* 1200 ml.) had cooled, it was acidified to *p*H 2–3 with concentrated HCl and extracted seven times with 200-ml. portions of ethyl ether. The ether solution was concentrated to 50 ml. and placed on an alumina column (Alcoa activated alumina, grade F-1) 7 inches high and 1 inch outside diameter. The column was eluted with ether until the ether became colorless and then eluted with 200 ml. of a 20% solution of absolute ethanol in ethyl ether. The ethanol–ether solution was evaporated to dryness and the residue recrystallized from water with the use of small amounts of charcoal as a decolorizing aid. After two recrystallizations, 14.5 mg. of pure "Factor A" was obtained, m.p. 154–155°, $\lambda_{\max}^{\mathrm{EtOH}}$ 230, 287 mμ, *E* 10,200, 6,785.

Anal. Calcd. for $C_8H_7NO_3$: C, 58.18; H, 4.29; N, 8.40; CH_3O-, 18.75. Found: C, 58.41; H, 4.27; N, 8.48; CH_3O-, 18.46.

The benzamide was prepared in pyridine solution and recrystallized from ethanol–water; m.p. 163–164°. *Anal.* Calcd. for $C_{15}H_{11}NO_4$: C, 66.92; H, 4.09; N, 5.20. Found: C, 66.93; H, 4.15; N, 5.18.

The benzenesulfonamide was prepared in the usual manner and recrystallized from ethanol–water; m.p. 163–164°. *Anal.* Calcd. for $C_{14}H_{11}NSO_5$: C, 55.08; H, 3.68; N, 4.59. Found: C, 54.72; H, 3.52; N, 4.44. "Factor A" was found to have a mol. wt. of 171 by boiling point elevation (calcd. 165).

Hydrolysis of "Factor A."—"Factor A" (100 mg.) was suspended in 10 ml. of water, flushed with nitrogen, and 10 ml. of 10% aqueous sodium hydroxide was added. The solution was refluxed for 3 hr. and the nitrogen allowed to pass through for an additional hour. The pale yellow solution was acidified to congo red with hydrochloric acid (deep red color) and extracted with ether. The ethereal solution yielded a crystalline unreacted "Factor A." The aqueous solution was evaporated to dryness leaving a purple residue which was extracted with hot ethanol to yield a crystalline compound which was identical with the hydrochloride of 2-amino-5-methoxyphenol (prepared synthetically) by infrared comparison. The purple color is apparently due to partial oxidation of the aminophenol.

6-Methoxybenzoxazolinone.—The hydrochloride of 2-amino-4-methoxyphenol[8] (6 g., 0.034 mole) was mixed with 3 g. (0.05 mole) of urea in an erlenmeyer flask equipped with an air condenser and the mixture heated in an oil-bath at 180° for 2 hr. After cooling to room temperature, the residue in the flask was washed with dilute acid and the insoluble material was dissolved in absolute alcohol. The aqueous acid was extracted with ether several times and the combined ether extracts evaporated to dryness. This residue was also dissolved in absolute alcohol. The alcohol solution was evaporated to a small volume and placed on an alumina column. The column was eluted with ethanol and a yellow crystalline product was obtained, m.p. 149–151°, 1.5 g. (26%). Recrystallization from water gave a white crystalline compound, m.p. 154–155°, which did not depress the melting point when mixed with "Factor A."

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[5] H. I. Virtanen and P. K. Hietala, *Acta Chem. Scand.*, **9**, 1954 (1955).

[6] H. I. Virtanen, P. K. Hietala and O. Wahlroos, *Suom. Kemist.*, **29B**, 143 (1956).

[7] E. E. Smissman, J. B. LaPidus and S. D. Beck, *J. Org. Chem.*, **22**, 220 (1957). (b) While our note was in the process of publication Hietala reported the synthesis of 6-methoxybenzoxazolinone by a different method: P. K. Hietala and O. Wahlroos, *Acta Chem. Scand.*, **10**, 1196 (1956).

[8] H. Bauer, *Ber*, **48**, 514(1915).