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### Isolation of an Alkaloid, Annuloline, from the Roots of *Lolium Multiflorum*<sup>1,2</sup>

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The roots of the annual rye grass (*Lolium multiflorum*) have been known for some time to contain a brilliant blue fluorescing pigment.<sup>5</sup> Seed analysts are able to distinguish annual rye grass seed from the morphologically similar perennial variety (*L. perenne*) by viewing the roots of the 8-day old seedlings under long wave ultraviolet light; the perenne roots do not fluoresce.<sup>6</sup>

The extraction of the fresh roots of the seedlings obtained from about 100 lbs. of seed of *L. multiflorum* has enabled us to isolate and crystallize the fluorescent principle which appears to be a weakly basic alkaloid. The tentative empirical formula, C<sub>20</sub>H<sub>19</sub>NO<sub>4</sub>, best satisfies the presently available data. It seems likely that there are three methoxyl groups present per formula weight.

A variety of organic solvents such as ethanol, methylene chloride, carbon tetrachloride, ether, benzene, and petroleum ether serve to extract this substance. Since it is both an extremely weak base and only sparingly soluble in water it cannot be readily extracted with hydrochloric acid. Having no acidic (or phenolic) groups it is not extractable with alkali. The pigment is strongly fluorescent. In petroleum ether it fluoresces with an intensity about 13 times that of quinine sulfate in 0.1 *N* sulfuric acid on a weight basis when compared in a model 12B Coleman fluorophotometer fitted with B-1 and PC-1 filters. It can be readily detected on filter paper by virtue of its fluorescence. In general it exhibits high mobilities on being chromat-

graphed on paper with solvents of low water content. Acidification of solvents with hydrochloric acid or basification with ammonium hydroxide such as employed by Swain<sup>7</sup> as diagnostic procedures for discriminating between acidic (or enolic) and basic plant pigments did not influence the mobility of the pigment.

A hydrochloride can be obtained by the action of anhydrous hydrogen chloride on a solution of the alkaloid in ethanol or petroleum ether. The resulting salt is strongly acidic. Because the characteristic blue fluorescence of solutions of the alkaloid is not shown by the salt form it is possible to estimate from a plot of fluorescence vs. *pH* that the base is approximately 50% in the free form at *pH* 2.0.

By means of ultraviolet light as little as 0.01 mcg. of the alkaloid may be detected when applied to a 2-mm. diameter area on Whatman No. 1 filter paper. Exposure of the spot to fumes of hydrochloric acid abolishes the blue fluorescence and the spot now glows yellow-green. In sufficient amounts the hydrochloride is visible as a green-yellow spot in visible light. Fumes of ammonia reverse this change. This simple test serves to distinguish the alkaloid from a number of naturally occurring fluorescent substances such as methyl anthranilate, anthranilic acid, terthienyl, various coumarins, flavanoids, and alkaloids.

A characteristic property of this alkaloid is the marked decrease in fluorescence which occurs in its solution in petroleum ether on the addition of various oxygen-containing solvents such as methanol, ethanol, *n*-propanol, ether, and acetone. Ethanol is remarkably effective in this respect. However its presence does not significantly alter the ultraviolet absorption spectrum as obtained with a petroleum ether solution of the alkaloid. The possibility that the quenching phenomenon is due to the high dielectric constants of the materials used is unlikely since a concentration of 0.12*M* of methanol is sufficient to cause a 50% loss of fluorescence in petroleum ether.

Workers in New Zealand<sup>8-12</sup> have isolated and characterized an alkaloid from the aerial portion of *Lolium perenne* (perennial rye grass) which they have named, perloline. Following this precedent we offer the name "annuloline" for the alkaloid isolated from the roots of the annual rye grass. Perloline and annuloline are clearly different compounds on the basis of their chemical and physical properties, analyses, fluorescent colors, absorption spectra,

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(2) A portion of this work is taken from the thesis submitted by J. R. B. in partial fulfillment of the requirements for the M.S. Degree, Purdue University, August, 1957.

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(5) G. Gentner, *Prakt. Blätter Pflanzenbau u. Pflanzenschutz*, **6**, 166 (1929).

(6) U. S. Dept. of Agriculture, *Testing Agricultural and Vegetable Seeds*, Government Printing Office, Washington, D. C. (1952), pp. 103-104.

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(9) R. E. R. Grimmet and D. F. Waters, *New Zealand J. Sci. Technol.*, **24B**, 151 (1943).

(10) I. Reifer and N. O. Bathurst, *New Zealand J. Sci. Technol.*, **24B**, 155 (1943).

(11) F. B. Shorland, *New Zealand J. Sci. Technol.*, **24B**, 159 (1943).

(12) E. M. Clare and I. M. Morice, *New Zealand J. Sci. Technol.*, **27B**, 36 (1945).

and distribution. We have been unable to detect any annuloline in plants of *Lolium perenne*.

#### EXPERIMENTAL

**Annuloline hydrochloride.** Seed of *Lolium multiflorum* were germinated in lots of 350 grams on 47 × 51 cm. sheets of Whatman No. 1 filter paper. Water was supplied daily. The germination temperature varied between 22–25°. After 17 days the aerial portions of the seedlings were discarded and the roots together with the filter paper in which they were matted were extracted with petroleum ether (b.p. 62°–65°). Extraction was performed using 7–10 l. of solvent for each lot of roots and allowed to proceed for at least 24 hr. (with occasional stirring). The extraction was repeated 2–3 times with fresh solvent. The combined extracts from 2–3 lots of roots were concentrated to about 200–300 ml. After being stored for several days at –20° the concentrate was filtered through glass wool in order to remove the waxy solid which deposited. The concentrate was further reduced in volume to about 50 ml. and treated with anhydrous HCl whereupon crude annuloline hydrochloride was obtained as a yellow-gray ppt. The hydrochloride was triturated with about 5 ml. of ice cold absolute ethanol, the mixture centrifuged at 2° and the supernatant was discarded. The air dried product was further purified by trituration with 10–25 ml. of H<sub>2</sub>O and extraction with a sufficient number of 50-ml. portions of petroleum ether to remove the alkaloid which was now in the form of the free base. The petroleum ether solution was concentrated to 50–100 ml. and treated with HCl gas as before. The hydrochloride was washed with cold absolute ethanol and dried, m.p. 174–177°.

*Anal.*<sup>13</sup> Calcd. for C<sub>20</sub>H<sub>20</sub>NO<sub>4</sub>Cl: C, 64.30; H, 5.38; N, 3.75; Cl, 9.5. Found: C, 64.89; H, 5.28; N, 3.57; Cl, 8.57, 9.02.

**Annuloline.** The hydrochloride was converted to the free base by shaking its CHCl<sub>3</sub> solution with dilute alkali. The free base was obtained as a brown tar on removal of the CHCl<sub>3</sub>. After prolonged cooling in an ice bath and stirring, followed by overnight storage at room temperature, the material became transformed into a microcrystalline yellow powder. Recrystallization from hot benzene to which petroleum ether had been added to produce incipient cloudiness yielded fragile, light-yellow narrow rectangles, occurring mainly in clusters; m.p. 105–106°, remelting at same temperature. The product was neutral and tasteless but possessing a pleasant spicy resinous odor. The ultraviolet spectrum in cyclohexane showed a maximum at 354 mμ (log ε = 4.48) and a minimum at 285 mμ (log ε = 3.85).

*Anal.* Calcd. for C<sub>17</sub>H<sub>10</sub>NO(OCH<sub>3</sub>)<sub>3</sub>: C, 71.20; H, 5.64; N, 4.15; (OCH<sub>3</sub>)<sub>3</sub>, 27.6. Found: C, 70.59; H, 5.65; N, 4.16; OCH<sub>3</sub>, 26.1.

The methoxyl determinations<sup>14</sup> were made by the titrimetric method described by Niederl and Niederl.<sup>15</sup> Since recovery of OCH<sub>3</sub> from vanillin standards was 92% of theory, the value shown for annuloline has been adjusted accordingly. Dioxymethylene groups were shown to be absent by the procedure of Gaebel.<sup>16</sup>

**Annuloline picrate.** A 1% ethanolic solution of annuloline hydrochloride was treated with a slight excess of a 1% ethanolic solution of picric acid. The yellow fibrous crystals were recrystallized from hot 80% ethanol, m.p. 216–218°. One mole of picric acid per atom of N was found colorimetrically.

(13) Elementary analyses were performed by Huffman Microanalytical Laboratories, Wheatridge, Colo.

(14) The methoxyl and dioxymethylene tests were carried out by Mr. R. S. Karimoto of this laboratory.

(15) J. B. Niederl and V. Niederl, *Organic Quantitative Micro-analysis*, J. Wiley and Sons, Inc., New York (1938), pp. 187–193.

(16) G. O. Gaebel, *Arch. Pharm.*, **248**, 226 (1910).

*Anal.* Calcd. for C<sub>20</sub>H<sub>19</sub>NO<sub>4</sub>·C<sub>6</sub>H<sub>3</sub>N<sub>3</sub>O<sub>7</sub>: C, 55.10; H, 3.89; N, 9.89. Found: C, 55.18; H, 3.86; N, 10.05.

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### The Neopentyl and Neophyl Systems in Peracid Oxidation of Ketones<sup>1</sup>

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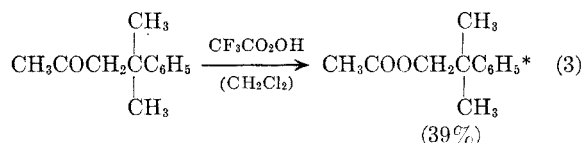
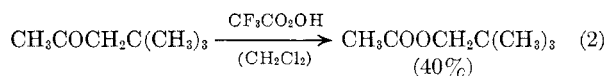
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The suggestion of Emmons and Lucas<sup>2</sup> that the migrating group in the Baeyer-Villiger ketone oxidation<sup>3</sup> normally is the one most stabilized by hyperconjugative electron release appears to be substantiated by their work. Thus, in all the alkyl methyl ketones they reported, the methyl group was never observed to migrate, while the other alkyl group present in each case was capable of such hyperconjugative electron release and gave an acetate ester, as indicated below.



The yields were excellent (mainly 70–90%).

We have investigated *neopentyl* methyl ketone and *neophyl* ( $\beta$ -phenylisobutyl) methyl ketone in this reaction, following closely the directions of Emmons and Lucas. These ketones give *neopentyl* and *neophyl* acetates, as shown below, with no detectable amounts of methyl or other alkyl esters.



\* Yield based on consumed starting ketone.

The yields are poorer, however, than those noted in Emmons' work.

Because the neopentyl and neophyl systems have *no* hyperconjugative electron release (indeed, these systems often rearrange to the tert-amyl and benzyldimethylcarbinyl systems in order to achieve such hyperconjugative stabilization), we believe the present work shows that this hyperconjugative ability is not necessary for migration. It seems from all the work reported thus far that *any* alkyl group migrates preferentially to methyl. Hyperconjugative electron release may, nevertheless, be im-

(1) Abstracted from the thesis of Albert Danielzadeh to be submitted to the Graduate School of Loyola University for the degree of Master of Science, February 1958.

(2) W. D. Emmons and G. B. Lucas, *J. Am. Chem. Soc.*, **77**, 2287 (1955).

(3) A. Baeyer and V. Villiger, *Ber.*, **32**, 3625 (1899) and other references to be found in the article cited in the previous footnote.