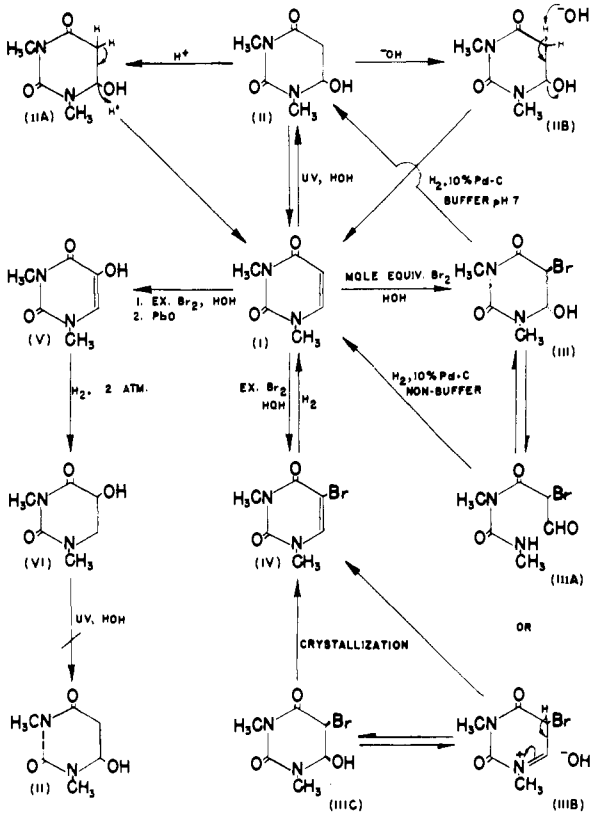
ULTRAVIOLET IRRADIATION OF 1,3-DIMETHYLURACIL[1]

Sir:

1,3-Dimethyluracil (I)[2] was used to study the interesting but poorly understood ultraviolet irradiation effects on nucleic acids.[3] (I) (m.p. 121–122°; $\lambda_{max}^{H_2O}$ 267 mμ, ε 8.67 × 10³; $\lambda_{min}^{H_2O}$ 235 mμ, ε 1.68 × 10³; λ250/λ260, λ280/λ260; 0.62, 0.62 for *p*H 2–12; ν_{CO}^{KCl} 1701 cm.$^{-1}$) in aqueous solution (0.067*M*) was irradiated with ultraviolet light until 80–90% of the optical density at 260 mμ had disappeared. The irradiated product (60–75% yield) was recrystallized from chloroform and petroleum ether and gave 6-hydroxy-1,3-dimethylhydrouracil (II),[4] m.p. 105–106° (found: C, 45.61; H, 6.49; N, 18.00; ν_{OH}^{KCl} 3344 cm.$^{-1}$, ν_{CO}^{KCl} 1704 cm.$^{-1}$). On treating (II) with acid (*p*H 2), alkali (*p*H 9, 10, 11, 12) or heat, respectively, the ultraviolet spectrum of (I) was reconstituted (λ250/λ260, λ280/λ260; 0.62, 0.62; 0.61, 0.62; 0.60, 0.62; 0.62, 0.63; 0.61, 0.62). Over 90% yield of (I) was obtained in the acidic and alkaline reconstitutions.



On treating (I) with an excess of bromine water, 5-bromo-1,3-dimethyluracil (IV), m.p. 184–185°, was obtained (found: C, 33.03; H, 3.40; N, 12.66; Br, 36.20; $\lambda_{max}^{H_2O}$ 283 mμ, ε 8.57 × 10³; $\lambda_{min}^{H_2O}$ 246 mμ, ε 1.57 × 10³; ν_{CO}^{KCl} 1684 cm.$^{-1}$). When (I) was treated with excess bromine water, followed by boiling with lead oxide, 5-hydroxy-1,3-dimethyluracil (V) m.p. 198–199° (found: C, 46.15; H, 5.38; N, 17.83; $\lambda_{max}^{H_2O}$ 285 mμ, ε 7.35 × 10³; $\lambda_{min}^{H_2O}$ 248 mμ, ε 1.78 × 10³; ν_{OH}^{KCl} 3215 cm.$^{-1}$, ν_{CO}^{KCl} 1669 cm.$^{-1}$) was obtained. Hydrogenation of (V) at two atmospheric pressures gave 5-hydroxy-1,3-dimethylhydrouracil (VI), m.p. 109–110° (found: C, 45.57; H, 6.40; N, 17.92; ν_{OH}^{KCl} 3390 cm.$^{-1}$, ν_{CO}^{KCl} 1704 cm.$^{-1}$). No reconstitution of (VI) under similar conditions. When (VI) was irradiated, then treated with acid or alkali, there was no change in ultraviolet spectrum, indicating that (VI) was not converted to (II) on irradiation. These observations suggested strongly that (VI) was not an intermediate.

When (I) was treated with one mole equivalent of bromine water *trans* 5-bromo-6-hydroxy-1,3-dimethylhydrouracil (III) was obtained in solution. There was no detectable change in ultraviolet spectrum after standing for two hours. However, when the solution was allowed to stand overnight or crystallized from the $CHCl_3$ extract, only (IV) was obtained. This dehydration probably went by way of the "mutarotation" (IIIA) or (IIIB). Hydrogenolysis of (III) in a non-buffered solution gave only (I) as the product. However, hydrogenolysis of (III) in a phosphate buffered solution, *p*H 7, gave (II), m.p. 105–106° in over 50% yield. This was identical with the irradiated product as determined by m.m.p. 105–106°, infrared and analysis (found: C, 45.56; H, 6.49; N, 17.71). (II) was thus identified by synthesis as 6-hydroxy-1,3-dimethylhydrouracil, suggesting a 1,4-addition as the mechanism of the photo-reaction.

The kinetics of the photo-addition were zero order as shown by a plot of optical density *vs.* time. The acid catalyzed dehydration (*p*H 1.98) (IIA), and the base catalyzed β-elimination (*p*H 9.01) (IIB) were both of the first order as shown by a plot of log C_0/C_t *vs.* time.

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(1) This work was done under the terms of Contract No. AT(30-1)-911 of the Physiology Department, Tufts University School of Medicine with the Atomic Energy Commission.

(2) A. M. Moore and C. H. Thomson, *Science*, **122**, 594 (1955), and references therein.

(3) E. Chargaff and J. N. Davidson, "The Nucleic Acids," Vol. I, p. 123, Academic Press, Inc., New York, N. Y.

(4) The product of Moore and Thomson, m.p. 102°. Following the nomenclature used in *Chemical Abstracts* (II) should be called 1,3-dimethyl-5,6-dihydro-6-hydroxy-2,4-pyrimidinedione.

THE BASIC RING SYSTEM OF CRININE

Sir:

Two recent communications have suggested the likelihood that several alkaloids of the *Amaryllidaceae* possess structures containing a spiro ring system.[1,2] The following results establish a new spiro ring system for crinine.[3]

Manganese dioxide oxidation of crinine, $C_{15}H_{14}N(O_2CH_2)(OH)$, m.p. 210°, gave an α,β-unsaturated ketone, oxocrinine (m.p. 183–185°; found: C, 71.15; H, 5.52; N, 5.21; $\lambda_{max}^{CHCl_3}$ 5.98 μ; λ_{max}^{EtOH} 227 mμ (4.20), 296 mμ (3.59)). Lithium aluminum hydride reduction of oxocrinine afforded *epi*crinine

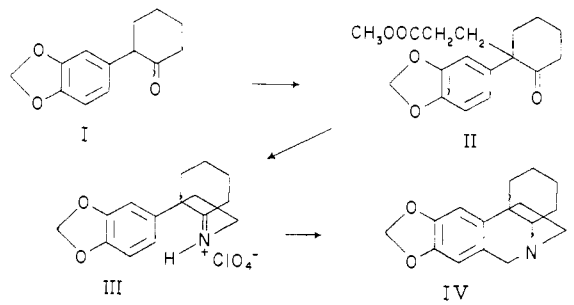
(1) S. Kobayashi, T. Shingu and S. Uyeo, *Chemistry and Industry* 177 (1956).

(2) T. Ikeda, W. I. Taylor, Y. Tsuda and S. Uyeo, *ibid.*, p. 411.

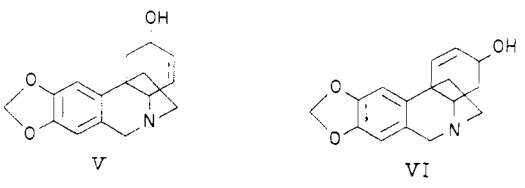
(3) Crinine was reported by L. H. Mason, E. R. Puschett and W. C. Wildman, THIS JOURNAL, **77**, 1253 (1955); in *Chem. Ber.*, **87**, 1704 (1954), H.-G. Boit reported the same alkaloid as "crinidine."

(m.p. 209–209.5°; found: C, 70.89; H, 6.31; N, 5.35). Dihydroöxocrinine (m.p. 157–159°; found: C, 70.96; H, 6.17; N, 5.12; $\lambda_{max}^{CHCl_3}$ 5.84; λ_{max}^{EtOH} 237 mμ (3.56), 294 mμ (3.72)), obtained by the catalytic hydrogenation of oxocrinine, formed a dibenzylidene derivative (m.p. 125°; found: C, 80.31; H, 5.88). Under mild Wolff–Kishner conditions,[4] dihydroöxocrinine gave *l*-crinane (5,10b-ethano-8,9-methylenedioxy-1,2,3,4,5,6,10b,10c-octahydrophenanthridine), (b.p. 130° (1 μ); $[\alpha]^{24}D$ −12.7°; found: C, 74.57; H, 7.63; N, 5.37); picrate (m.p. 206–207°; found: C, 54.40; H, 4.52; N, 11.51). *l*-Crinane also was obtained from the catalytic hydrogenation-hydrogenolysis of crinine.

dl-Crinane was synthesized in the following manner. 2-(3,4-Methylenedioxyphenyl)-cyclohexanone (m.p. 93–94°; found: C, 71.33; H, 6.55) was obtained from 5-(3,4-methylenedioxyphenyl)-4-nitrocyclohexene[5] by the Nef reaction.[6] Cyanoethylation of I and subsequent methanolysis of the nitrile gave II (m.p. 88–89°; found: C, 67.15; H, 6.51). The action of nitrous acid on the hydrazone hydrazide[7] of II gave 2,3,4,5,6,7-hexahydro-3a-(3,4-methylenedioxyphenyl)-indole which was isolated as the hydroperchlorate (III), (m.p. 227–229°; found: C, 52.51; H, 5.27; Cl, 10.14). Catalytic hydrogenation of III and Pictet–Spengler cyclization of the resultant base afforded *dl*-crinane (IV), (m.p. 97–99°; found: C, 74.69; H, 7.27; N, 5.41); picrate (m.p. 218–220°; found: C, 54.49; H, 4.35; N, 11.60). The infrared spectra (liquid film) of the *l*-crinane and the synthetic crinane were superimposable, as were the spectra of the natural and synthetic picrates in chloroform solution.



Crinine must be represented by V or VI. A 3-hydroxy-Δ^{4-4a} structure is unlikely since dihydrocrinine (pK_a 8.70) is only slightly more basic than crinine (pK_a 7.95).[8] The stability of dihydrooxocrinine to base and its methohydroxide to the Hofmann degradation favors structure V.



Consistent with the morphine-like skeleton of these alkaloids, several exhibit significant analgesic activity.[9]

TABLE I

PRELIMINARY RESULTS ON ANALGESIC ACTIVITY OF AMARYLLIDACEAE ALKALOIDS AND THEIR DERIVATIVES

Alkaloid	ED_{50}	LD_{50}	Duration min.
Buphanidrine	6.2	8.9	106
dl-Crinane	20	40	...
Crinine	45	~100	...
Galanthamine	2.7	11.3	141
Lycoramine	21.3	89	133
Morphine	2.1	576	129

(9) I am indebted to Dr. Nathan B. Eddy, National Institute of Arthritis and Metabolic Diseases, for the pharmacological data.

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RECEIVED JULY 16, 1956

STUDIES ON ADRENOCORTICOTROPIN. XIII. THE ISOLATION OF TWO HIGHLY ACTIVE BOVINE TYPES

Sir:

By means of chromatography on the carboxylic type ion exchange resin XE-97[1] we have separated two highly potent types of adrenocorticotropin from beef pituitary extracts. As shown in Fig. 1, the two active types (A_1 and A_2) emerge from the column as clearly separated retarded peaks.[2] The distinction between the two peaks in terms of hold-up volumes is maintained when the peptide material recovered from each peak is re-run separately through the same column. The salt-free peptide products recovered[3] from the peaks show potencies in the USP test of about 85 units per milligram by the intravenous method. This value is slightly lower than those obtained for the purest porcine[4,5] and sheep[6] preparations, but is several times higher than the best potencies yet obtained for fractions isolated from beef sources.[7]

The starting material used in our columns was an oxycellulose eluate fraction made from crude extracts of beef anterior glands by a process similar to that described by Astwood, *et al.*[8] As noted by others,[7] our oxycellulose eluates from beef extracts have lower potencies than those from pork by a

(1) Rohm and Haas Co., Washington Square, Philadelphia 5, Pa.

(2) Although we had previously (in our work with pork ACTH) numbered our chromatographic peaks in the order of their emergence from the column, we are here adopting the reverse convention used by Dixon and Stack-Dunne in their recent paper (*cf.* reference 10).

(3) For de-salting the column fractions, we have used two methods with equal success: (a) three 45 minute dialyses against 10 volumes of 0.1 *N* acetic acid, or (b) distribution to phenol and back again to water. Our procedure in the latter method is similar to that of Dixon and Stack-Dunne (*cf.* reference 10) except that we use 0.02 *N* acetic acid as the aqueous phase and lyophilize the final solution directly.

(4) W. F. White, THIS JOURNAL, **75**, 503 (1953).

(5) P. H. Bell, *ibid.*, **76**, 5565 (1954).

(6) C. H. Li, I. I. Geschwind, J. S. Dixon, A. L. Levy and J. I. Harris, *J. Biol. Chem.*, **213**, 171 (1955).

(7) W. T. Koch and F. J. Wolf, THIS JOURNAL, **77**, 489 (1955).

(8) E. B. Astwood, M. S. Raben, R. W. Payne and A. B Grady, *ibid.*, **73**, 2969 (1951).

(4) M. Gates and G. Tschudi, THIS JOURNAL, **78**, 1380 (1956).

(5) L. H. Mason and W. C. Wildman, *ibid.*, **76**, 6194 (1954).

(6) W. C. Wildman and R. B. Wildman, *J. Org. Chem.*, **17**, 581 (1952).

(7) W. E. Bachmann and E. J. Fornefeld, THIS JOURNAL, **73**, 51 (1951).

(8) V. Prelog and O. Häfliger, *Helv. Chim. Acta*, **32**, 1851 (1949).