dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated. The amorphous residue (5 mg.) showed absorption at 3.65, 5.62 and 5.80 μ.

Protoverine 6-Acetate 15-(*l*)-2′-Methylbutyrate (XI).—A solution of protoverine 15-(*l*)-2′-methylbutyrate 6,16-diacetate (540 mg.), m.p. 217–219° dec., in methanol (15 ml.) was allowed to stand at room temperature for 11 hours. The methanol was evaporated under reduced pressure and the residue was chromatographed on Merck acid-washed alumina (10 g.). The column yielded to chloroform a resin (60 mg.). A paper chromatogram[10] indicated that this resin was mainly starting material. The column yielded to 1% methanol–chloroform a resin (230 mg.) which was crystallized from chloroform–petroleum ether as needles (200 mg.), m.p. 238–239° dec. Two recrystallizations from chloroform–petroleum ether gave fine colorless needles (150 mg.), m.p. 248–249° dec., $[\alpha]^{24}D$ −23° (*c* 1.01, pyr.).

Anal. Calcd. for $C_{34}H_{53}O_{11}N\cdot CHCl_3$: C, 54.51; H, 7.06. Found: C, 54.79; H, 7.02.

In a volatile acid determination,[12] 11.97 mg. of the compound yielded an amount of acid equivalent to 3.15 ml. of 0.009375 *N* sodium thiosulfate; calcd. for 1 mole of acetic acid and 1 mole of (*l*)-2-methylbutyric acid, as expected for structure XI, 3.31 ml.

A 10-mg. sample of this compound was oxidized with sodium periodate under conditions described for a sodium periodate titration.[8] After 2 hours, the solution was basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated. The amorphous residue (5.5 mg.) showed absorption at 3.65, 5.62 and 5.80 μ.

Conversion of Desacetylprotoveratrine B to Protoverine 6-Acetate 15-(*l*)-2′-Methylbutyrate.—A solution of desacetylprotoveratrine B (250 mg.), m.p. 201–203° dec., in 5% acetic acid (6 ml.) was treated with a solution of periodic acid (250 mg.) in a mixture of water (3 ml.) and *t*-butyl alcohol (18 ml.). The solution was allowed to stand at room temperature for 1 hour, the excess of the oxidizing agent was destroyed by rapid addition of 0.1 *N* aqueous sodium arsenite (25 ml.) and the solution was extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was chromatographed on Merck acid-washed alumina (8 g.). The column yielded to chloroform and to 2% methanol–chloroform, a yellow oil; to 5% methanol–chloroform, a white solid. The solid was crystallized from acetone as chunky rods (85 mg.), m.p. 241–242° dec. The compound was recrystallized from chloroform–petroleum ether as fine needles (62 mg.), m.p. 248–249° dec. The m.p. was not depressed on admixture with protoverine 6-acetate 15-(*l*)-2′-methlybutyrate. The paper chromatographic behavior[10] and infrared spectra (potassium bromide pellet) of the respective samples were identical.

Conversion of Desacetylprotoveratrine A to Protoverine 6-Acetate 15-(*l*)-2′-Methylbutyrate.—A solution of desacetylprotoveratrine A (130 mg.), m.p. 201–202° dec., and sodium borohydride (45 mg.) in a mixture of pyridine (10 ml.) and *t*-butyl alcohol (10 ml.) was allowed to stand at room temperature for 30 minutes. The excess of the borohydride was destroyed by addition of acetic acid (5 ml.), the solution basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was chromatographed on Merck acid-washed alumina (7 g.). The column yielded to chloroform and to 2% methanol–chloroform a starting material (55 mg.); to 5% methanol–chloroform, a resin. The resin was crystallized from chloroform–ether as needles (20 mg.). A paper chromatogram[10] indicated that the product was slightly impure. Two recrystallizations from chloroform–petroleum ether effectively purified the material and afforded colorless needles (6 mg.), m.p. 244–247° dec. The m.p. was not depressed on admixture with protoverine 6-acetate 15-(*l*)-2′-methylbutyrate. The paper chromatographic behavior[10] and infrared spectra (potassium bromide pellet) of the respective samples were identical.

MADISON, WISC.

[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

The Alkaloids of *Nerine bowdenii* W. Wats. and *Crinum moorei* J. D. Hook.[1]

BY R. E. LYLE,[2a] E. A. KIELAR, J. R. CROWDER[2b] AND W. C. WILDMAN

RECEIVED OCTOBER 21, 1959

The seeds of *Crinum moorei* J. D. Hook. have been found to contain the alkaloids lycorine, crinamidine, powelline, crinine and 1-acetyllycorine. 1-Acetyllycorine had not been isolated previously from natural sources. The bulbs of *Nerine bowdenii* W. Wats. have been shown to contain at least sixteen alkaloids, four of which have not been reported to date. The method of isolation is described in detail, and the new alkaloids from both sources are characterized. One of the new alkaloids was identified as (+)-epicrinine (III). The conversion of (+)-epicrinine to (±)-crinane is reported.

In the course of studies in this Laboratory on the structures of undulatine[3] and crinamidine,[4] we sought plant materials which would provide these alkaloids in quantity and at reasonable cost. The isolation of these alkaloids from the bulbs of *Nerine undulata* (L.) Herb.,[4] *N. flexuosa* Herb.,[5] *N. bowdenii* W. Wats.[6] and *Crinum moorei* Hook. f.[7] has been reported. Of these plant sources, only the latter two are relatively abundant in this country, and the yields of undulatine and crinamidine from them were reported to be less than 0.01%. Although we were unable to find plants that were appreciably richer in these bases, a number of interesting new alkaloids were discovered in the course of the exploratory studies.

Through the courtesy of Mr. N. F. Giridlian,[8] we were able to purchase quantities of the seeds of *Crinum moorei* J. D. Hook. In addition to the expected alkaloids crinamidine, powelline, lycorine and crinine, a new alkaloid, $C_{18}H_{19}NO_5$, was obtained, m.p. 220–221°, $[\alpha]^{23.5}_{589}$ −96° (chloroform). Degradative evidence, which will be presented later in this paper, established the alkaloid as 1-acetyllycorine (I, R = CH_3CO, R_1 = H).

The isolations from *N. bowdenii* were considerably more complex. In an earlier study of the alkaloids of this bulb, Boit and Ehmke[6] reported the

(1) Paper XV of a series on Amaryllidaceae alkaloids; previous paper: P. F. Highet and W. C. Wildman, *J. Org. Chem.*, in press.

(2) Visiting Scientist, National Heart Institute: (a) 1958–1959, (b) 1956–1957.

(3) E. W. Warnhoff and W. C. Wildman, *Chemistry & Industry*, 1293 (1958).

(4) H.-G. Boit, *Chem. Ber.*, **89**, 1129 (1956).

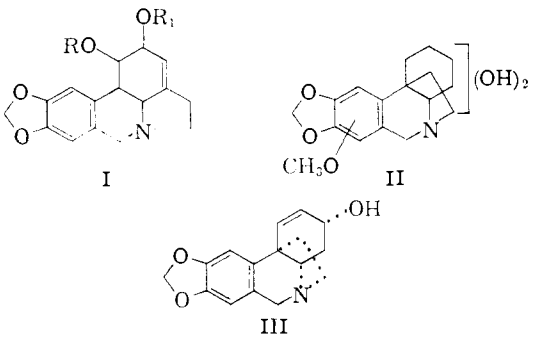
(5) H.-G. Boit and H. Ehmke, *ibid.*, **90**, 369 (1957).

(6) H.-G. Boit and H. Ehmke, *ibid.*, **89**, 2093 (1956).

(7) H.-G. Boit, *ibid.*, **87**, 1704 (1954).

(8) Oakhurst Gardens, Arcadia, Calif.

presence of ambelline, crinamidine, crinine, lycorine and undulatine. They noted that these crystalline alkaloids represented a remarkably low percentage of the extractable crude alkaloids. Although we have isolated sixteen alkaloids from this plant source, 66% of the total alkaloid fraction still remains non-crystalline and uncharacterized. Our method of isolation involved a preliminary fractionation of the alkaloids into bases forming chloroform-soluble and chloroform-insoluble hydrochlorides.[9] In th former group were found most of



the non-hydroxylic alkaloids and a majority of the ambelline, while the latter group contained the hydroxylic bases and buphanisine. Isolation of the pure alkaloids was achieved by a combination of direct crystallization and chromatography on Florisil or alumina. A summary of these isolations is given in Table I, and the specific methods are described in the Experimental section.

TABLE I

ALKALOIDS OF *Nerine bowdenii* W. WATS.

Alkaloid	Weight, g.[a]	Yield, %	Compound or structure ref.
1-Acetyllycorine	0.36	0.0017	10
Ambelline	10.87	.051	11
Belladine	12.6[b]	.059	12
Bowdensine	2.88	.014	10
Buphanidrine	3.65	.017	13
Buphanisine	0.26	.0012	13
Crinamidine	2.28	.011	5, 14
Crinamine	0.71	.0033	15
Crinine	2.74	.013	13
(+)-Epicrinine	1.63	.0077	10
Lycorine	9.55	.045	16
Nerbowdine	0.47	.0022	10
Undulatine	5.87	.028	3

[a] Isolated from 47 pounds of fresh *Nerine bowdenii* bulbs. [b] Calculated as free base from weight of alkaloid hydrochloride.

1-Acetyllycorine, which was isolated both from the seeds of *C. moorei* and the bulbs of *N. bowdenii*, showed normal methylenedioxyphenyl absorption at 236 and 293 mμ. The infrared spectrum showed bands at 2.81, 5.76, 9.60 and 10.65 μ, indicating the presence of hydroxyl, carbonyl and methylenedioxy groups, respectively.[17] The alkaloid was identified as an O-acetyllcorine since further acetylation gave O,O-diacetyllcorine (I, R, R_1 = $COCH_3$), while reductive cleavage of the acetyl group with lithium aluminum hydride afforded lycorine (I, R, R_1 = H). Both 1- and 2-acetyllcorine have been synthesized from lycorine,[18,19] and a comparison of the new alkaloid with 1-acetyllycorine (I, R = CH_3CO, R_1 = H) that had been prepared by the partial hydrolysis of O,O-diacetyllycorine[18] showed the two materials to be identical.

Nerbowdine had been isolated earlier in this Laboratory from unidentified *Brunsvigia* and *Boöphone* species of South African origin. This alkaloid, $C_{17}H_{21}NO_5$, $[\alpha]^{23}_{589}$ −109°, is polymorphic, crystallizing from chloroform–acetone as prisms, m.p. 230–232°, and from ethanol to give a form melting with decomposition at 242–245°. The infrared spectra in chloroform of the two polymorphs were identical, but they differed considerably when run as Nujol mulls. Nerbowdine formed crystalline salts with hydrochloric and nitric acids and gave a crystalline O,O-diacetate. The two hydroxyl groups of nerbowdine were found to be non-vicinal. Analytical data indicated that the base contained one methoxyl group but no N-methyl. From the hydrocotarnine-like ultraviolet absorption spectrum of the base and the occurrence of infrared absorption at 6.16, 9.6 and 10.65 μ, it seems probable that the aromatic ring is substituted with methylenedioxy and methoxyl groups.[17,20] Nerbowdine absorbed no hydrogen under catalytic conditions. From the expanded molecular formula, $C_{15}H_{14}N(OH)_2(O_2CH_2)(OCH_3)$, the characteristic powelline-like infrared spectrum and the structures of its companion alkaloids, nerbowdine would appear to be a dihydroxypowellane (II).[21]

A mixture of bowdensine and buphanidrine was eluted after undulatine in the chromatographic separations of the alkaloids forming chloroform-soluble hydrochlorides. These bases could be separated either by selective precipitation of buphanidrine picrate from an ethanolic solution of the mixture or by saponification of the mixture and isolation of the chloroform-insoluble deacetylbowdensine. Deacetylbowdensine, $C_{17}H_{21}NO_5$, was isolated in trace amounts from the chromatographic separation of

(9) *Cf.* J. Renz, D. Stauffacher and E. Seebeck, *Helv. Chim. Acta*, **38**, 1209 (1955).

(10) This paper.

(11) L. H. Mason, E. R. Puschett and W. C. Wildman, THIS JOURNAL, **77**, 1253 (1955).

(12) E. W. Warnhoff, *Chemistry & Industry*, 1385 (1957).

(13) W. C. Wildman, THIS JOURNAL, **80**, 2567 (1958).

(14) Ref. 5 reports the structure of crinamidine as a hydroxypowelline; in a later paper of this series, crinamidine will be shown to be 1,2-epoxypowelline.

(15) H. M. Fales and W. C. Wildman, THIS JOURNAL, **82**, 197 (1960).

(16) L. G. Humber, H. Kondo, K. Kotera, S. Takagi, K. Takeda, W. I. Taylor, B. R. Thomas, Y. Ysuda, K. Tsukamoto, S. Uyeo, H. Yajima and N. Yanaihara, *J. Chem. Soc.*, 4622 (1954).

(17) L. H. Briggs, L. D. Colebrook, H. M. Fales and W. C. Wildman, *Anal. Chem.*, **29**, 904 (1957).

(18) Y. Nakagawa, S. Uyeo and H. Yajima, *Chemistry & Industry*, 1238 (1956).

(19) K. Takeda, K. Kotera and S. Mizukami, THIS JOURNAL, **80**, 2562 (1958).

(20) W. C. Wildman and C. J. Kaufman, *ibid.*, **77**, 4807 (1955).

(21) Nerbowdine bears a superficial resemblance to haemanthine[22] in melting point behavior, optical rotation and functional groups. However, no correlation appears to exist between the physical properties of the respective derivatives of the alkaloids or in the molecular formula.

(22) A. N. Bates, J. K. Cooke, L. J. Dry, A. Goosen, H. Krüsi and F. L. Warren, *J. Chem. Soc.*, 2537 (1957).

the chloroform-insoluble hydrochlorides. It seems most probable that the deacetylbowdensine isolated in this manner was an artifact produced by the hydrolysis of bowdensine during the isolation process. Bowdensine, $C_{21}H_{25}NO_7$, $[\alpha]^{24}_{589}$ +17.3°, is not crystalline but forms a well-defined hydroperchlorate, m.p. 260–262° dec., and a methiodide, m.p. 284–285° dec. Analytical data show the presence of one methoxyl and two acetyl groups. The latter are not vicinal, since deacetylbowdensine was found not to be a vicinal glycol. No hydrogen was absorbed when either bowdensine or deacetylbowdensine was treated with hydrogen in the presence of palladium-on-charcoal. The spectral evidence offered earlier for the type of aromatic substitution in nerbowdine also is applicable to bowdensine and its deacetyl derivative, and it would appear that deacetylbowdensine is a hydroxy isomer or epimer of nerbowdine.

(+)-Epicrinine was separated from crinine by fractional crystallization from chloroform–acetone in which (+)-epicrinine is much less soluble. Its infrared spectrum in chloroform solution and in a Nujol mull was identical with that of (−)-epicrinine prepared by the lithium aluminum hydride reduction of oxocrinine.[13] The melting points of the two substances were the same (209–210°), but on admixture the melting point was raised to more than 230°. The rotations of (+)-epicrinine and (−)-epicrinine[13] were of equal magnitude but opposite in sign. When equal weights of the two substances were mixed and recrystallized from chloroform–acetone, (±)-epicrinine was obtained, m.p. 239°, $[\alpha]^{24}_{589-400}$ ±0.0°. Oxidation of (+)-epicrinine with manganese dioxide gave (+)-oxocrinine which showed all the properties expected of the enantiomorph of oxocrinine.

The basic nucleus of crinine was established first through a comparison of the liquid film infrared spectra of (−)-crinane that had been obtained from crinine and synthetic (±)-5,10b-ethano-8,9-methylenedioxy - 1,2,3,4,4a,5,6,10b - octahydrophenanthridine.[13] Since these compounds showed a sufficient number of bands in the infrared to establish identity, resolution of the synthetic material was not pursued. With the availability of (+)-epicrinine, the degradation of (±)-oxocrinine to (±)-crinane became feasible. By combining equal weights of (+)- and (−)-oxocrinine, a sufficient quantity of (±)-oxocrinine was obtained to degrade the racemic mixture to (±)-crinane. The (±)-crinane obtained from (±)-oxocrinine was identical in melting point and infrared spectrum (KBr and chloroform) with synthetic (±)-crinane, and a mixture melting point determination showed no depression.

(+)-Epicrinine is the second alkaloid to be isolated from plants of the Amaryllidaceae which is elaborated from the (+)-crinane nucleus and is unsubstituted at C_{11}. Previously, the isolation of vittatine, the optical antipode of crinine, had been reported.[5] Chemical evidence showing that haemanthamine, haemanthidine, crinamine and haemultine are derived from (+)-crinane has been presented in brief form.[23]

(23) W. C. Wildman and H. M. Fales, THIS JOURNAL, 80, 6465 (1958).

Experimental[24]

Isolations from *Nerine bowdenii*.—The fresh bulbs, 47 pounds, were ground twice in a Hobart grinder and stirred for 45 minutes at 50–55° with 35 gal. of 95% ethanol. The material was allowed to stand at room temperature for two days. The supernatant liquid was removed by a siphon. The solid plant material was then stirred with 35 gal. of 95% ethanol at 50–60° for 45 minutes and allowed to cool overnight. The mixture was filtered through gauze, and the ethanolic solutions from the two extractions were concentrated to 4.6 l. in a circulating evaporator. This concentrate was diluted with two volumes of water, acidified with 300 ml. of 2 *N* hydrochloric acid, and filtered through Celite. The Celite cake was washed once with 200 ml. of 2 *N* hydrochloric acid and once with water, and the filtrates were added to the original aqueous filtrate. The aqueous solution was extracted twice with 3.5 l. of chloroform in a Super Centactor model AC-1.[25] The chloroform extract was concentrated under reduced pressure to give 107 g. of neutral material and alkaloids forming chloroform-soluble hydrochlorides. The aqueous solution was basified with concentrated ammonium hydroxide to *p*H 10 and extracted four times in the Centactor with 4-l. portions of chloroform. Concentration of this chloroform extract gave 8.0 g. of lycorine which precipitated and was removed by filtration. Further concentration of the chloroform solution gave 88.2 g. of crude alkaloids forming chloroform-insoluble hydrochlorides.

The aqueous solution still contained alkaloidal material and was extracted in a continuous chloroform extractor overnight. Concentration of the chloroform solution gave 6.2 g. of dark brown resin. This material was triturated with methanolic chloroform to give a small quantity of insoluble lycorine. The crude lycorine was purified through its hydrochloride salt, 1.11 g., m.p. 210–212° dec. The soluble filtrates were not investigated further.

The chloroform wash (107 g.) was treated with five 200-ml. portions of 2 *N* hydrochloric acid to extract the alkaloids from the neutral materials. The combined aqueous solutions were treated with Darco, filtered and washed five times with chloroform. These chloroform washes were combined and shaken with an excess of ammonium hydroxide. The ammoniacal aqueous solution was extracted twice with chloroform and added to the chloroform solution which was concentrated under reduced pressure to yield 73 g. of alkaloidal material forming chloroform-soluble hydrochlorides. The acidic aqueous solution was basified with ammonium hydroxide and extracted exhaustively with chloroform. These chloroform extracts were concentrated to yield 13 g. of alkaloidal material forming chloroform-insoluble hydrochlorides.

The chloroform extract (88.2 g.) and the 13 g. of crude alkaloids forming chloroform-insoluble hydrochlorides were combined and dissolved in warm (50°) dilute hydrochloric acid, treated with Darco and filtered. The acid solution was extracted three times with chloroform. The chloroform extract was washed with ammonium hydroxide, then water, and concentrated to afford 5.44 g. of crude alkaloids forming chloroform-soluble hydrochlorides which were added to the 73 g. of similar material mentioned above. The aqueous acid solution was basified with ammonium hydroxide and extracted exhaustively with chloroform. Concentration of the combined chloroform solutions gave 65 g. of alkaloids forming chloroform-insoluble hydrochlorides.

Both the alkaloid fraction (78.44 g.) forming chloroform-soluble hydrochlorides and that (65 g.) forming chloroform-insoluble hydrochlorides were divided into three approximately equal parts for chromatography. Although each part may not have been treated in exactly the same manner, in the interests of brevity, only the most successful isolation procedure for each fraction is described below. The yields shown in Table I are based on material actually obtained rather than as a factor of best procedure.

(24) All melting points were observed on a Kofler microscope hot-stage and are corrected. The boiling points are uncorrected. Unless otherwise noted, rotations were measured on a Rudolph photoelectric polarimeter using a 2-dm. tube, and ultraviolet spectra were obtained in absolute ethanol solution on a Cary model 11 MS recording spectrophotometer. Infrared spectra were recorded on a Perkin–Elmer model 21 double-beam spectrophotometer, in chloroform solution unless noted to the contrary. Analyses were performed by Mr. J. F. Alicino, Metuchen, N. J.

(25) The Sharples Corporation, Bridgeport, Pa.

Alkaloids Forming Chloroform-soluble Hydrochlorides.—A solution of approximately 18.0 g. of crude alkaloids forming chloroform-soluble hydrochlorides in 50 ml. of absolute ethanol was saturated with gaseous hydrogen chloride, then diluted with ether to the point of permanent turbidity. The flask was seeded with belladine hydrochloride and set in the refrigerator for two days. The precipitate was filtered and recrystallized twice from ethanol to give 3.8 g. of **belladine hydrochloride**, m.p. 190–192° (reported[26] 190–192°). The filtrates were diluted with water, basified with ammonium hydroxide and extracted with chloroform. Concentration of the chloroform extracts gave 10.67 g. of alkaloids which was diluted with ethyl acetate and seeded with ambelline. The solution was allowed to stand overnight, and the precipitated **ambelline**, 600 mg., m.p. 210–240° dec., was removed by filtration and recrystallized from chloroform–acetone to give 480 mg. of pure ambelline, m.p. 253–256° dec. (reported[11] 260–261° dec.). The filtrate was concentrated under reduced pressure, dissolved in benzene and chromatographed on 300 g. of Florisil. Elution with 50% ethyl acetate in benzene afforded first a belladine-containing forerun, followed by **undulatine** which was crystallized from ethanol–water to give 775 mg., m.p. 149–150° (reported m.p. 151–152°,[3] 148–149°[5]). Further elution with this mixture and with ethyl acetate gave considerable quantities of alkaloids containing an ester function as shown by infrared spectra. Further elution with increasing percentages of methanol (up to 20%) gave no additional crystalline compounds.

Alkaloids Forming Chloroform-insoluble Hydrochlorides.—A chloroform solution containing approximate 19 g. of alkaloids was chromatographed on 600 g. of Florisil packed in chloroform. After elution of a trace of non-alkaloidal material, 3% methanol in chloroform eluted a large fraction from which 1.70 g. of **ambelline**, m.p. 252–255° dec., was obtained by crystallization from ethyl acetate. Further elution with this solvent gave fractions from which 610 mg. of **crinamidine**, m.p. 228–230°, was obtained by crysallization from chloroform–acetone. Elution with 10% methanol in chloroform gave fractions from which was isolated 628 mg. of **crinine**, m.p. 208–210° (reported[11] m.p. 209–210°), after recrystallization from acetone. From these later fractions there was isolated 202 mg. of **lycorine** which was separated easily by virtue of its insolubility in acetone. Elution with 50% methanol–chloroform afforded a large amount of amorphous material from which 126 mg. of **nerbowdine**, m.p. 232–235°, was obtained by trituration with acetone.

All alkaloidal material eluted before undulatine in the chromatography of the alkaloids forming chloroform-soluble hydrochlorides was dissolved in ethanol, saturated with hydrogen chloride and seeded with belladine hydrochloride. The precipitated material was recrystallized from ethanol to give 1.60 g. of pure **belladine hydrochloride**, m.p. 195–196°. Upon rechromatographing all undulatine filtrates, there was obtained first 443 mg. of **undulatine**, m.p. 149–150°. Further elution produced a large amount of ester-containing oils which were not crystalline. Saponification of this material and separation of the resultant substances into base hydrochlorides soluble and insoluble in chloroform, followed by crystallization and/or chromatography gave 1.092 g. of **ambelline**, m.p. 255–260° dec., and 460 mg. of **undulatine**, m.p. 148–150°. Because of the insoluble nature of ambelline, it is highly unlikely that it existed as such in the ester mixture prior to saponification. The ease of elution of these fractions and the infrared spectrum of the crude ester mixture lend support to the fact that no unacylated ambelline was present in these fractions. No proof of the size of the acylating group was attempted, but in view of the number of acetylated alkaloids present in *N. bowdenii*, the acetyl group is most likely.

The large quantities of ester-containing alkaloids which were eluted after undulatine were dissolved in ethanol and treated with ethanolic picric acid. The precipitated picrate was recrystallized from either chloroform–ethanol or acetone to give **buphanidrine picrate**, m.p. 240° (reported[13] m.p. 238–239°). All picrate filtrates were combined and converted to the free alkaloids by alkali and extraction with chloroform. The crude buphanidrine picrate was treated in the same manner. A solution of the recovered buphanidrine in acetone was acidified with 60% perchloric acid. The precipitated **buphanidrine hydroperchlorate** was recrystallized twice from acetone–ether to give 2.32 g. of pure salt, m.p. 247–249° (reported m.p. 250–252°,[13] 240–242°[9]). Treatment of the alkaloids forming soluble picrates in the same manner gave a total of 4.00 g. of **bowdensine hydroperchlorate** as an acetone solvate, m.p. 260–262°. The filtrates from each perchlorate were combined; the free bases were regenerated, and buphanidrine and bowdensine again were separated through the picrate and perchlorate salts. After this procedure had been completed a second time, there remained 1.7 g. of alkaloidal material which was a mixture of undulatine and buphanidrine. Chromatography on alumina first afforded 452 mg. of crude **undulatine** which was recrystallized from ethanol–water to give 239 mg. of pure material, m.p. 149–150°. This was followed by 500 mg. of crude **buphanidrine**, purified *via* its hydroperchlorate, 339 mg., m.p. 250–252° dec.

A chloroform solution of 18.1 g. of ambelline filtrates was rechromatographed on 550 g. of Florisil. Elution with 2% methanolic chloroform gave a mixture of buphanisine and 1-acetyllycorine which was separated by rechromatography on alumina to give **buphanisine**, 261 mg., m.p. 124–125°, $[\alpha]^{24}_{589}$ −24° (*c* 1.07, ethanol) (reported m.p. 125–126°, $[\alpha]^{25}_{D}$ −24°;[27] m.p. 122–124°, $[\alpha]^{20}_{D}$ −26°[9]) after recrystallization from ether, and **1-acetyllycorine**, 360 mg., m.p. 219–221° after recrystallization from ethyl acetate. Further elution with 2% methanol in chloroform gave fractions containing **crinamine** which were combined and crystallized from acetone to give 706 mg., m.p. 196–197° (reported[11] m.p. 198–199°). Elution with chloroform containing higher percentages of methanol gave a number of oily fractions from which 140 mg. of **ambelline**, m.p. 253–256° dec., was obtained.

A chloroform solution of approximately 7.4 g. of filtrates from the crinamidine fractions was passed over 280 g. of Florisil packed in chloroform. Elution with 2% methanol in chloroform gave first **ambelline**, 322 mg., m.p. 257–260° dec., and then **crinamidine**, 613 mg., m.p. 229–230°. Elution with more polar solvents gave no additional crystalline material.

A solution of approximately 12.0 g. of crinine fraction filtrates was rechromatographed on 250 g. of Florisil packed in chloroform. Elution with 3% methanol in chloroform gave 2.0 g. of an ester-containing oil. Further elution with this solvent and with 10% methanol in chloroform gave crystalline mixtures of crinine and (+)-epicrinine. Separation was effected by recrystallization from chloroform–acetone, in which the latter is much less soluble. This method afforded 1.27 g. of **(+)-epicrinine**, m.p. 207–209°, and 780 mg. of **crinine**, m.p. 208–210°.

Isolation of Alkaloids from *Crinum moorei*.—By a procedure identical with that described for *N. bowdenii*, 6455 g. of *C. moorei* seeds afforded 15.1 g. of alkaloids forming chloroform-insoluble hydrochlorides and 1.8 g. of alkaloids forming chloroform-soluble hydrochlorides. The former fraction was treated with 200 ml. of chloroform and allowed to stand overnight. The precipitated lycorine, 1.70 g. (0.026%), was removed by filtration and washed with chloroform. The chloroform filtrates were washed three times with 10% sodium hydroxide, twice with saturated brine and once with water. Concentration of the chloroform solution gave 12.6 g. of basic, non-phenolic alkaloids. From the aqueous alkaline solutions there was isolated 0.5 g. of phenolic alkaloids which were not investigated further. The 12.6-g. alkaloid fraction was dissolved in chloroform and chromatographed on 350 g. of silicic acid packed in chloroform. Elution with 5% methanol in chloroform gave 4.0 g. of oil from which 2.50 g. (0.039%) of **1-acetyllycorine**, m.p. 220–221°, was obtained by crystallization from acetone. Further elution with this mixture afforded fractions from which there was isolated 1.40 g. (0.022%) of **crinamidine**, m.p. 232–233°, by trituration and recrystallization from acetone. Elution with 15% methanol in chloroform gave 820 mg. (0.013%) of **powelline**, m.p. 198–199° (reported[13] m.p. 197–198°) by the same technique. Finally, there was eluted by 30% methanol in chloroform, 530 mg. (0.0084%) of **crinine**, m.p. 207–208° after recrystallization from acetone.

The isolation and characterization of ambelline, buphanidrine, buphanisine, crinamine, crinine, lycorine, powelline and undulatine have been described in previous papers of this series. In the current isolations, identification was effected by comparison of melting points, mixed melting points and infrared spectra.

(26) A. R. Surrey, A. Mooradian, R. A. Cutler, C. M. Suter and J. S. Buck, THIS JOURNAL, **71**, 2421 (1949).

(27) H. M. Fales and W. C. Wildman, *ibid.*, **80**, 4395 (1958).

1-Acetyllycorine (I, $R = COCH_3$, $R_1 = H$).—The pure alkaloid crystallized from acetone as colorless prisms, m.p. 220–221°, $[\alpha]^{23}_{589}$ −96°, $[\alpha]^{23}_{436}$ −212° (*c* 1.11, chloroform); $[\alpha]^{24}_{589}$ −66.0°, $[\alpha]^{24}_{436}$ −149° (*c* 1.18, ethanol); λ^{EtOH}_{max} 236 mμ (ε 3950) and 293 mμ (ε 4800).

Anal. Calcd. for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.81; H, 5.99; N, 4.17; OCH_3, 0.0; NCH_3, 0.0.

A sample of 1-acetyllycorine prepared from 0,0-diacetyllycorine by the method of Uyeo,[18] m.p. 221–222°, $[\alpha]^{24}_{589}$ −96°, $[\alpha]^{24}_{436}$ −212° (*c* 1.04, chloroform); $[\alpha]^{23.5}_{589}$ −66°; $[\alpha]^{23.5}_{436}$ −151° (*c* 1.04, ethanol), was identical in infrared spectrum (chloroform) with the natural material and a mixture melting point determination showed no depression.

O,O-Diacetyllycorine.—A solution of 204 mg. of 1-acetyllycorine in 2 ml. of pyridine and 2 ml. of acetic anhydride was allowed to stand overnight at room temperature. The reaction mixture was poured into 10% sodium carbonate solution and the product was extracted with chloroform. The solvent was removed under reduced pressure, and the residue was crystallized from acetone to yield 134 mg. of colorless prisms, m.p. 220–221°, $[\alpha]^{23.5}_{589}$ +25° $[\alpha]^{23.5}_{436}$ +48° (*c* 1.00, chloroform). This material showed no depression in melting point when mixed with authentic 0,0-diacetyllycorine, m.p. 221–222°, $[\alpha]^{23.5}_{589}$ +25°, $[\alpha]^{23.5}_{436}$ +50° (*c* 1.15, chloroform) [reported m.p. 217–218.5°, $[\alpha]^{26}_{589}$ + 26.8° (chloroform),[28] m.p. 219–221°, $[\alpha]^{26}_{589}$ +26.6°[29]] prepared from lycorine.

Lycorine.—A solution of 350 mg. of 1-acetyllycorine in 30 ml. of tetrahydrofuran was refluxed with 0.6 g. of lithium aluminum hydride for 18 hours. The reaction mixture was hydrolyzed with ethyl acetate and alkali. Concentration of the organic layers gave a solid which was recrystallized from ethanol, 233 mg., m.p. 250–260° dec. The infrared spectrum (Nujol) was identical with that of lycorine.

(+)-Epicrinine.—The alkaloid was purified by recrystallization from chloroform–acetone and sublimation at 170° (1 μ); m.p. 209–210°, $[\alpha]^{22}_{589}$ +136°, $[\alpha]^{22}_{436}$ +329° (*c* 1.03, chloroform) [reported[13] for (−)-epicrinine: m.p. 209–209.5°, $[\alpha]^{27}_{589}$ −142°, $[\alpha]^{27}_{436}$ −343°].

Anal. Calcd. for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 70.97; H, 6.24; N, 5.27; OCH_3, 0.0.

(±)-Epicrinine.—Equal weights of (+)- and (−)-epicrinine were recrystallized from chloroform-acetone and finally sublimed under reduced pressure; m.p. 239°, $[\alpha]^{24}_{589-400}$ 0.0° (*c* 1.17, chloroform).

Anal. Found: C, 70.61; H, 6.25; N, 5.23.

(+)-Oxocrinine.—By the method described for (−)-oxocrinine,[13] there was obtained from 50 mg. of (+)-epicrinine, 40 mg. of (+)-oxocrinine, m.p. 187–188°, $[\alpha]^{22}_{589}$ +319°, $[\alpha]^{22}_{436}$ +804° (*c* 1.09, chloroform) [reported[13] for (−)-oxocrinine: m.p. 184–186°, $[\alpha]^{24}_{589}$ −307°, $[\alpha]^{24}_{436}$ −848°].

Anal. Calcd. for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.37; H, 5.64; N, 5.30.

(±)-Oxocrinine.—A chloroform solution of 91 mg. each of (+)- and (−)-oxocrinine was concentrated to a gum, crystallized from ethanol and then sublimed; 143 mg., m.p. 177–178°, $[\alpha]^{25}_{589-350}$ 0.0° (*c* 1.26, ethanol).

Anal. Found: C, 71.29; H, 5.72; N, 5.31.

(±)-Dihydrooxocrinine.—By the procedure described for the preparation of (−)-dihydrooxocrinine,[13] there was obtained from 435 mg. of (±)-oxocrinine 305 mg. of product, m.p. 174–175°, $[\alpha]^{25}_{589-350}$ 0.0° (*c* 1.03, ethanol).

Anal. Calcd. for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 70.77; H, 6.45; N, 5.19.

(±)-Crinane.—Wolff–Kishner reduction of 400 mg. of (±)-dihydrooxocrinine by the procedure described earlier[13] for (−)-dihydrooxocrinine gave 352 mg. of oil. Trituration with ether and recrystallization from ether gave a total of 160 mg. of (±)-crinane, m.p. 110–111°.

Anal. Calcd. for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.70; H, 7.34; N, 5.48.

(±)-Crinane prepared by synthesis, m.p. 97–99°, was found to be identical in infrared spectrum (chloroform) with that prepared by degradation. When a sample of synthetic (±)-crinane was sublimed at 100° (10 μ), the higher melting polymorph, m.p. 110–111°, was obtained. When the (±)-crinane, m.p. 97–99°, was heated to 105° and seeded with this higher melting polymorph, the melt crystallized and remelted at 110–111°. Synthetic (±)-crinane, m.p. 110–111°, and (±)-crinane obtained by degradation showed no mixture melting point depression and possessed identical infrared spectra in Nujol and KBr. A mixture of (±)-crinane and (−)-crinane, m.p. 109–110°, melted at 96–108°.

Crinamidine.—The pure base formed fine needles from acetone, m.p. 232–233°, $[\alpha]^{27}_{589}$ −7°, $[\alpha]^{27}_{436}$ −10° (*c* 0.6, chloroform) [reported[7] m.p. 235–236° dec., $[\alpha]^{22}_{D}$ −24° (*c* 0.6, chloroform)]; λ^{EtOH}_{max} 288 mμ (ε 1585).

Anal. Calcd. for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41; OCH_3, 9.78; neut. equiv., 317. Found: C, 64.36; H, 5.91; N, 4.52; OCH_3, 9.92; neut. equiv., 316.

Crinamidine Picrate.—Prepared in ethanol and recrystallized from aqueous ethanol, crinamidine picrate formed yellow prisms, m.p. 131–132°.

Anal. Calcd. for $C_{17}H_{19}NO_5 \cdot C_6H_3N_3O_7 \cdot H_2O$: C, 48.94; H, 4.29; N, 9.93. Found: C, 49.03; H, 4.15; N, 9.95.

Crinamidine Methiodide.—Prepared in acetone and recrystallized from water, the methiodide formed colorless prisms, m.p. 263–265° dec. (reported[7] m.p. 265° dec.).

Anal. Calcd. for $C_{17}H_{19}NO_5 \cdot CH_3I$: C, 47.07; H, 4.83; I, 27.63. Found: C, 47.03; H, 5.21; I, 27.50.

Nerbowdine.—The base crystallized from chloroform–acetone to give prisms, m.p. 230–232°. Recrystallization from ethanol gave a different polymorph, m.p. 244–245° dec. A sample was sublimed at 180° (10 μ) for analysis, m.p. 230–232°, $[\alpha]^{23}_{589}$ −108.8° (*c* 0.97, chloroform), λ^{EtOH}_{max} 287 mμ (ε 1620).

Anal. Calcd. for $C_{17}H_{21}NO_5$: C, 63.93; H, 6.63; N, 4.39; OCH_3, 9.72. Found: C, 63.86; H, 6.65; N, 4.32; OCH_3, 9.42; vicinal glycol, 0.00.

An ethanolic solution of nerbowdine absorbed no hydrogen in the presence of 10% palladium-on-charcoal at room temperature and atmospheric pressure.

Nerbowdine Hydrochloride.—Prepared in dilute aqueous hydrochloric acid and recrystallized from aqueous ethanol, the hydrochloride formed fine needles, m.p. 254–265° dec., $[\alpha]^{23}_{589}$ −86.1°, $[\alpha]^{23}_{436}$ −180° (*c* 0.87, water).

Anal. Calcd. for $C_{17}H_{22}NO_5Cl$: C, 57.38; H, 6.23. Found: C, 57.24; H, 6.11.

Nerbowdine Hydronitrate.—Prepared by the addition of a solution of sodium nitrate to an aqueous acetic acid solution of nerbowdine, the hydronitrate formed fine needles from ethanol, m.p. 227–240° dec., $[\alpha]^{24}_{589}$ −81°, $[\alpha]^{24}_{436}$ −168° (*c* 1.06, water).

Anal. Calcd. for $C_{17}H_{22}N_2O_8$: C, 53.40; H, 5.80. Found: C, 53.52; H, 5.75.

0,0-Diacetylnerbowdine.—A solution of 164 mg. of nerbowdine in 1 ml. of pyridine and 2 ml. of acetic anhydride was allowed to stand 10 days at room temperature (convenience). The pyridine and acetic anhydride were removed under reduced pressure. A chloroform solution of the residue was washed twice with sodium bicarbonate and once with water. The chloroform solution was concentrated and chromatographed on 10 g. of aluminum oxide (Merck). Elution with 30–50% ethyl acetate in benzene gave 200 mg. of colorless oil which was distilled at 170° (5 μ). The distillate crystallized and was sublimed for analysis, m.p. 151–152°, $[\alpha]^{25}_{589}$ −30.5°, $[\alpha]^{25}_{436}$ −47.1° (*c* 1.05, chloroform).

Anal. Calcd. for $C_{21}H_{25}NO_7$: C, 62.52; H, 6.25; N, 3.47; 2 CH_3CO, 21.34. Found: C, 62.28; H, 6.20; N, 3.64; CH_3CO, 21.06.

Bowdensine Hydroperchlorate Acetone Solvate.—Recrystallization of the salt from acetone gave bowdensine hydroperchlorate as colorless prisms which underwent a transition at 160° and melted at 260–262° dec., $[\alpha]^{24}_{589}$ +5°, $[\alpha]^{24}_{436}$ +15° (*c* 0.4, 75% ethanol). The salt was shown to contain a molecule of acetone of solvation by analysis, infrared spectrum and positive test with 2,4-dinitrophenylhydrazine.

Anal. Calcd. for $C_{21}H_{26}NO_{11}Cl \cdot C_3H_6O$: C, 51.28; H, 5.74; mol. wt., 562. Found: C, 51.45; H, 5.84; mol. wt., 570.

Bowdensine.—A suspension of 247 mg. of bowdensine hydroperchlorate acetone solvate in 10 ml. of water was neutralized with 10% sodium hydroxide, and the aqueous

(28) W. C. Wildman and C. J. Kaufman, THIS JOURNAL, **76**, 5815 (1954).

(29) A. Hunger and T. Reichstein, *Helv. Chim. Acta*, **36**, 824 (1953).

solution was extracted with four 15-ml. portions of chloroform. The solution was concentrated and chromatographed on a 4-cm. column of alumina. Bowdensine, 212 mg., was obtained in the first 150 ml. of chloroform eluent. Two successive evaporative distillations at 140° (0.50 mm.) gave pure bowdensine as a colorless glass, $[\alpha]^{24}_{589}$ +17.3°, $[\alpha]^{24}_{436}$ +48.1° (*c* 1.06, chloroform).

Anal. Calcd. for $C_{21}H_{25}NO_7$: C, 62.52; H, 6.25; N, 3.47; OCH_3, 7.69; neut. equiv., 403. Found: C, 62.48; H, 6.50; N, 3.45; OCH_3, 7.94; neut. equiv., 406.

Bowdensine Methiodide.—A solution of 45.2 mg. of bowdensine in ether was treated with an excess of methyl iodide. The methiodide, 49.4 mg., which precipitated was recrystallized from ethanol to give 41.9 mg. of bowdensine methiodide, m.p. 284–285° dec., $[\alpha]^{24}_{589}$ +9.8°, $[\alpha]^{24}_{436}$ +25.5° (*c* 1.30, 90% ethanol).

Anal. Calcd. for $C_{22}H_{28}NO_7I$: C, 48.44; H, 5.17. Found: C, 48.40; H, 5.18.

Deacetylbowdensine.—A solution of 50.8 mg. of bowdensine hydroperchlorate acetone solvate in 5 ml. of absolute ethanol containing 95 mg. of potassium hydroxide was heated under reflux for 30 minutes. The solvent was removed by evaporation and the residue was treated with a small amount of distilled water. The precipitate was removed by centrifugation and washed twice with water. Upon drying there was obtained 28.8 mg. of deacetylbowdensine, m.p. 276–277° dec., $[\alpha]^{25}_{589}$ −43.7°, $[\alpha]^{25}_{436}$ −86.6° (*c* 0.5, ethanol). Extraction of the aqueous filtrates with chloroform provided an additional 7.2 mg. of deacetylbowdensine, m.p. 260–265° dec. Recrystallization from absolute ethanol gave analytically pure deacetylbowdensine, m.p. 277–278° dec., λ^{EtOH}_{max} 287 mμ (ε 1685).

Anal. Calcd. for $C_{17}H_{21}NO_5$: C, 63.93; H, 6.63; N, 4.39; OCH_3, 9.72; neut. equiv., 319. Found: C, 64.04; H, 6.58; N, 4.27; OCH_3, 9.59; neut. equiv., 310; vicinal glycol, 0.00.

A solution of 70.7 mg. of deacetylbowdensine in absolute ethanol absorbed no hydrogen when stirred with 10% palladium-on-charcoal for 24 hours, and 68.1 mg. of starting material, m.p. 268–270° dec., was recovered.

Bowdensine from Deacetylbowdensine.—A solution of 104 mg. of deacetylbowdensine in 30 ml. of pyridine and 10 ml. of acetic anhydride was allowed to stand at room temperature for 26 hours. The solvent was removed by distillation, and the residual oil was chromatographed on Florisil. Bowdensine was eluted by 5–10% absolute ethanol in chloroform to give 113 mg. of product which showed an infrared spectrum in chloroform that was identical with that of bowdensine that had been isolated directly. A portion of the 113 mg. was evaporatively distilled at 145° (0.05 mm.) to give analytically pure bowdensine, $[\alpha]^{25}_{589}$ +17°, $[\alpha]^{25}_{436}$ +48° (*c* 1.46, chloroform).

A portion of the crude bowdensine above in ether was neutrallized with 60% perchloric acid, and the resulting precipitate was recrystallized from acetone to give bowdensine hydroperchlorate acetone solvate, m.p. 255–256° dec., which gave an infrared spectrum and optical rotation, $[\alpha]^{25}_{589}$ +5°, $[\alpha]^{25}_{436}$ +15° (*c* 0.39, 75% ethanol), identical with those of the hydroperchlorate of the natural ester.

The methiodide was prepared from a portion of the bowdensine in ether. Recrystallization from absolute ethanol gave pure bowdensine methiodide, m.p. 287–288° dec., $[\alpha]^{24}_{589}$ +11°, $[\alpha]^{24}_{436}$ +28° (*c* 1.14, 90% ethanol), the infrared spectrum of which was identical with that of the methiodide of the natural ester.

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Poly-L-cyclohexylalanine and Poly-L-cyclohexylalanyl Proteins

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RECEIVED NOVEMBER 3, 1959

L-Cyclohexylalanine, prepared by the catalytic hydrogenation of L-phenylalanine, was allowed to react with phosgene to yield N-carboxy-L-cyclohexylalanine anhydride (III); III was polymerized in dioxane to give poly-L-cyclohexylalanine. A copolymer of L-cyclohexylalanine and L-glutamic acid was also synthesized. Poly-L-cyclohexylalanyl gelatins and poly-L-cyclohexylalanyl egg albumin, as well as a gelatin enriched with both L-cyclohexylalanine and L-glutamic acid, were obtained by making use of the amino groups of the proteins as initiators in the polymerization of N-carboxy-α-amino acid anhydrides.

Several amino acids occurring in proteins contain aromatic rings in their side chains. In studies concerning the contribution of various amino acids to specific biochemical functions of peptides and proteins, it seemed to us to be of interest to find out whether the aromatic character of the ring is necessary for the particular biological property investigated. For example, the replacement of phenylalanine by cyclohexylalanine would show the extent to which the property studied would change as a result of the saturation of the aromatic ring. Thus Jennings and Niemann[1] reported that α-chymotrypsin catalyzes the hydrolysis of acetyl-L-cyclohexylalaninamide in aqueous solutions at 25° and *p*H 7.9 at a rate equal to that of acetyl-L-phenylalaninamide. Edelson, *et al.*,[2] reported recently that cyclohexylalanine is not a phenylalanine antagonist in *Leuconostoc dextranicum* 8086.

In the course of an investigation of the chemical basis of the antigenicity of proteins[3,4] it was observed that the attachment of peptides of the aromatic amino acids tyrosine, phenylalanine and tryptophan converts gelatin into powerful antigens. In order to be able to elucidate the role of the aromatic character in the enhancement of the antigenicity of gelatin, it seemed desirable to investigate the immunological properties of derivatives of gelatin enriched with cyclohexylalanine.

The synthesis of N-carboxy-L-cyclohexylalanine anhydride (III), of poly-L-cyclohexylalanine (IV), of polycyclohexylalanyl gelatin and of other polymeric derivatives containing L-cyclohexylalanine is described in the present article. The results of the immunological studies will be reported elsewhere.

L-Cyclohexylalanine has been prepared by the catalytic hydrogenation of L-tyrosine[5-7] and of L-phenylalanine.[5] Shemin and Herbst[8] synthesized

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(6) P. Karrer and W. Kehl, *ibid.*, **13**, 50 (1930).

(7) J. H. Billman and J. A. Buehler, *Proc. Indiana Acad. Sci.*, **63**, 120 (1953).

(8) D. Shemin and R. M. Herbst, THIS JOURNAL, **61**, 2471 (1939).