seconds. After filtration and drying the yellow solid was leached with 60 ml. of hot acetone. This extract was concentrated to dryness and the residue was then leached with 20 ml. of acetone at 25°. The residue from evaporation of this extract was recrystallized twice from 6 ml. of ethanol to give 0.15 g. of yellow prisms, m.p. 241–247° (cloudy). This fraction (VII) was soluble in sodium bicarbonate and contained 17.47% N. Calcd. for $C_{16}H_9N_2O(NO_2)_3$: N, 18.27.

C.—To 0.5 g. of the hydroxypyrazine in 20 ml. of sulfuric acid at 0° was added over ten minutes 0.09 ml. of nitric acid (d. 1.5) in 10 ml. of sulfuric acid. After 15 minutes at 0° the solution was poured into 200 ml. of water and the yellow solid was filtered off. This was boiled a few minutes in 300 ml. of 1% aqueous sodium bicarbonate and after cooling to 25° the mixture was filtered. Acidification of the yellow filtrate precipitated a small amount of solid which was leached with 8 ml. of methanol at 25°. The residue from evaporation of the methanol was recrystallized from 4 ml. of benzene and then from 1.5 ml. of acetone to yield 6 mg. of large yellow granules, m.p. 237–240°. Presumably this is 2-hydroxy-5-nitro-3,6-diphenylpyrazine (VIII).

Anal. Calcd. for $C_{16}H_{11}N_3O_3$: N, 14.33. Found: N, 14.60.

BROOKLYN, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY, AND THE DEPARTMENT OF PHARMACOLOGY, HARVARD MEDICAL SCHOOL]

Schoenocaulon Alkaloids. I. Active Principles of *Schoenocaulon officinale*. Cevacine and Protocevine[1,2]

BY S. MORRIS KUPCHAN, DAVID LAVIE,[3] C. V. DELIWALA[4] AND B. Y. A. ANDOH

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On the basis of analogies drawn from the behavior of zygadenine and its esters upon treatment with alkali, the existence of an isomeric, carbonyl-free cevagenine precursor was postulated. In a search for such a precursor, the amorphous fraction of commercial veratrine after removal of cevadine and veratridine was investigated. Fractionation using chromatographic procedures led to isolation of a new alkamine, protocevine, $C_{27}H_{43}O_3N$, isomeric with cevagenine and cevine and a new ester alkaloid, cevacine, $C_{29}H_{45}O_9N$, a monoacetate ester of protocevine. Cevacine yields protocevine upon methanolysis, and protocevine is isomerized to cevagenine by mild alkaline treatment. Acetylation of protocevine with acetic anhydride and pyridine yields protocevine triacetate and similar acetylation of cevacine affords the same triester. Acetylation of protocevine with acetic anhydride and perchloric acid yields anhydroprotocevine tetraacetate, and cevadine has been shown to give an analogous tetraester (anhydrocevadine triacetate) under the same conditions. Protocevine can be obtained from cevadine by methanolysis or by alkaline hydrolysis under very mild conditions. Very mild alkaline hydrolysis of veratridine also yields protocevine. It is proposed that protocevine, and not cevagenine, is the parent alkamine of cevacine, cevadine and veratridine.

Commercial veratrine consists of a mixture of alkaloids obtained from the seeds of *Schoenocaulon officinale*, Gray. Two well characterized ester alkaloids, cevadine and veratridine, have been isolated from the mixture, and these esters yield angelic acid and veratric acid, respectively, on alkaline hydrolysis. Until recently, the alkamine present in cevadine and veratridine was generally believed to be cevine, since this was the only alkamine previously obtainable from the esters by hydrolysis.[5]

Stoll and Seebeck[5] showed, in 1952, that cevadine and veratridine yield cevagenine, a carbonyl-containing isomer of cevine, upon hydrolysis under mild alkaline conditions. They showed further that cevagenine is converted into cevine by treatment with strong alkali (20% alcoholic potassium hydroxide). On the basis of these facts and the evidence that both cevadine and cevagenine show carbonyl absorption at 5.86 μ, those authors concluded that cevagenine, not cevine, is the "genuine" alkamine of cevadine and veratridine.

In a recent paper[6] we have described the relationships between the esters veratroylzygadenine and vanilloylzygadenine and the alkamine zygadenine. Upon mild alkaline treatment, veratroylzygadenine and zygadenine are converted first to an amorphous carbonyl-containing alkamine isomeric with zygadenine. By stronger alkaline treatment, with alcoholic sodium hydroxide, the amorphous alkamine is converted to a second zygadenine isomer, pseudozygadenine, which is carbonyl-free.

The pharmacodynamic action of veratroylzygadenine is similar to that of veratridine.[7] There is also a resemblance between the infrared spectra of the two esters, particularly in the region of 9 to 11.5 μ. Furthermore, the respective products of strong alkaline treatment, pseudozygadenine and cevine, show characteristic and similar absorption properties in the 9 to 11.5 μ region (compare Fig. 1 with Fig. 1 in reference 6). These facts suggested a parallel between the two alkaloidal series, and led us to speculate that a carbonyl-free cevagenine precursor might exist, which would occupy a position in the schoenocaulon series analogous to that of zygadenine in the zygadenus series.

The contrasting behavior of cevadine and cevagenine upon acetylation[8] cast doubt upon the proposal that cevadine is an angelic acid ester of cevagenine. When cevagenine is treated with acetic anhydride and pyridine at steam-bath temperature, dehydration accompanies acetylation, and anhydrocevagenine triacetate is obtained. Treatment

(1) This work was supported by grants from the National Institutes of Health, Eli Lilly and Company, and the William T. Wellington Memorial Research Fund.

(2) Presented, in part, at the 123rd Meeting of the American Chemical Society, Los Angeles, Calif., March 17, 1953 (Abstracts, p. 29M).

(3) On leave of absence from the Weizmann Institute of Science, Rehovoth, Israel.

(4) Haffkine Institute, Bombay, India.

(5) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **35**, 1270 (1952). An excellent, concise summary of the literature on the hydrolysis of cevadine and veratridine is given in the introduction to this paper.

(6) S. M. Kupchan and C. V. Deliwala, THIS JOURNAL, **75**, 1025 (1953).

(7) O. Krayer, B. H. Rogers, S. M. Kupchan and C. V. Deliwala, *Federation Proc.*, **11**, 364 (1952).

(8) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **35**, 1942 (1952).

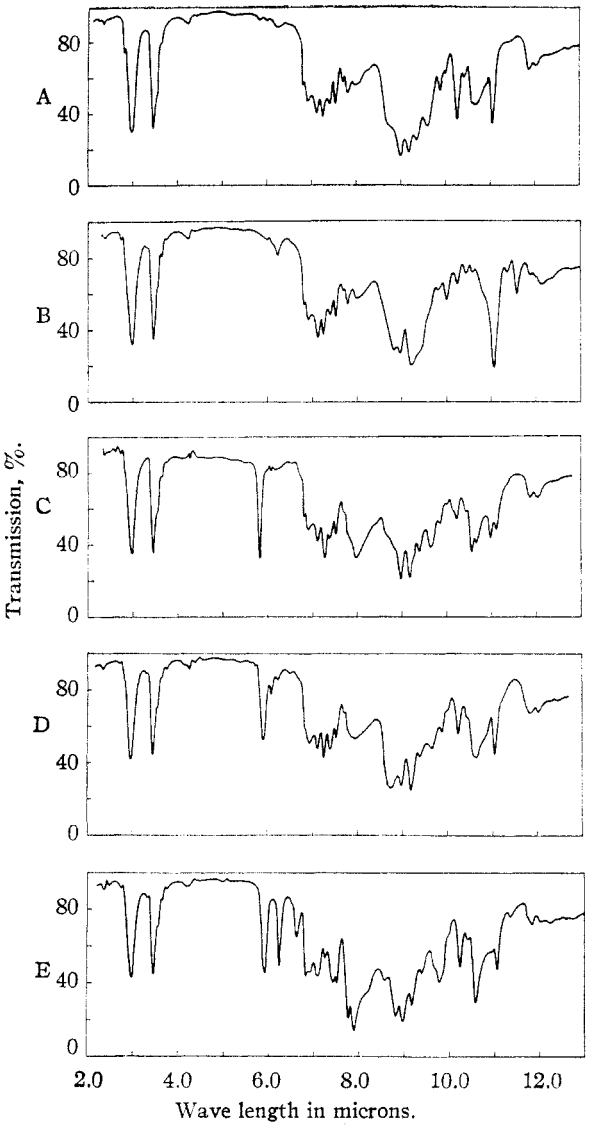


Fig. 1.—Infrared spectra in chloroform: A, protocevine; B, cevine; C, cevacine; D, cevadine; E, veratridine.

of cevadine under the same conditions leads only to acetylation, and the product is cevadine diacetate. This difference appeared to us to lend support to the possibility that the parent alkamine of cevadine and veratridine is not cevagenine, but a cevagenine precursor.

The current investigation of the alkaloidal components of commercial veratrine was undertaken with a view toward elucidation of the relationships between the schoenocaulon ester alkaloids and the alkamines of the series.

Our preliminary separation procedure was patterned on the method employed by Blount[9] for the isolation of cevadine and veratridine. Refinements in technique led to improvement in the yields of both ester alkaloids, and we have therefore described our fractionation procedure in some detail (see Experimental). Of the fractions remaining after removal of cevadine and veratridine, the material in the filtrate after removal of the insoluble nitrate salt received the closest scrutiny. The free bases in this filtrate were liberated by addition of aqueous ammonia and separated into three fractions. Fraction A was obtained by filtration of the precipitated solid. Fraction B was obtained by ether extraction of the filtrate from fraction A. Subsequent chloroform extraction of the same filtrate yielded fraction C.

Crystallization of fraction A from methanol–water yielded an additional crop of cevadine. Fractionation of B by chromatography on sulfuric acid-washed alumina gave a new crystalline ester alkaloid (I), m.p. 205–207°, $[\alpha]^{21}D$ −27° (*c* 2.67, chf.). From fraction C, a new alkamine (II) was obtained which softens to a semi-solid mass at 179–183° and then melts gradually up to 220–225° dec., $[\alpha]^{23}D$ −33° (*c* 2.13, chf.), $[\alpha]^{22}D$ −26° (*c* 1.80, alc.).

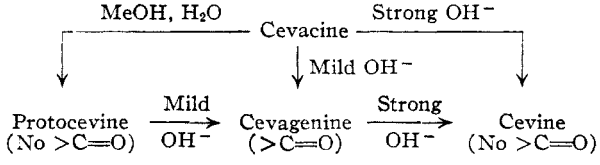
Analysis of the alkamine II gave values agreeing with the theoretical values for $C_{27}H_{43}O_3N$, an indication that the alkamine is isomeric with cevagenine and cevine. Mild alkaline treatment of II, under conditions comparable to those used in the conversion of cevadine to cevagenine,[5] yielded cevagenine. When II was heated with 20% alcoholic potassium hydroxide (the procedure used for hydrolysis of the schoenocaulon esters to cevine), cevine was obtained. Alkamine II, then, is the sought-after carbonyl-free precursor (*cf.* Chart I) of cevagenine and cevine, and has been named **protocevine**.

Acetylation of protocevine with acetic anhydride and pyridine yielded protocevine triacetate, m.p. 239–241° dec., $[\alpha]^{22}D$ −22° (*c* 1.63 chf.). When protocevine was treated with acetic anhydride and perchloric acid, dehydration accompanied acetylation, and the product obtained was anhydroprotocevine tetraacetate, m.p. 253–254° dec., $[\alpha]^{23}D$ +77° (*c* 1.90, alc.). Anhydroprotocevine tetraacetate was also obtained by treatment of protocevine triacetate with acetic anhydride and perchloric acid.

The new ester alkaloid I afforded analytical values for carbon, hydrogen and nitrogen which agree with the formula $C_{29}H_{45}O_9N$. Acetyl determination revealed the presence of one acetyl group in the molecule. Methanolysis of I yielded protocevine, and acetylation gave protocevine triacetate, identical in all respects with the acetylation product of protocevine. Hence I is a monoacetate ester of protocevine, for which we propose the name **cevacine**. Like protocevine, cevacine is converted into cevagenine by mild alkaline hydrolysis and into cevine by stronger alkaline treatment. The relationships between cevacine and the protocevine isomers are summarized in Chart I.

CHART I

RELATIONSHIPS BETWEEN CEVACINE AND THE PROTOCEVINE ISOMERS



Comparison of the infrared spectrum of cevadine diacetate with that of protocevine triacetate and of cevagenine triacetate suggested a far closer resem-

(9) B. K. Blount, *J. Chem. Soc.*, 122 (1935).

blance to the protocevine ester than to the cevagenine ester (*cf.* Fig. 2). The difference between the absorption of the cevagenine ester and those of the other two esters is particularly marked in the 10 to 11.5 μ range. This evidence suggested that protocevine, and not cevagenine, is the parent alkamine of cevadine, as well as of cevacine. Further support for this suggestion came from a study of the cleavage of cevadine and veratridine under mild conditions. Room-temperature methanolysis of cevadine for 5 days gave a small yield of protocevine. When a solution of cevadine in methanol–water was heated under reflux for 5 days, protocevine was obtained in 15–20% yield. Alkaline hydrolysis of cevadine and veratridine with very dilute methanolic alkali and a 10-minute heating period afforded protocevine (35–40% yield) along with cevagenine (15–20% yield).[10]

The reported behavior of cevadine upon acetylation with acetic anhydride and perchloric acid appeared to be a serious flaw in the argument that protocevine is the parent alkamine of this ester. Unlike protocevine, which, as shown above, gives an anhydro-tetraester under these conditions, cevadine has been reported to give an anhydro-triester (anhydrocevadine diacetate).[8] This anomaly has now been resolved by re-examination of the anhydro-acetate obtained from cevadine. By volatile acid determination using *p*-toluenesulfonic acid for the hydrolysis,[11] the anhydro-acetate has been shown to be a tetraester, anhydrocevadine triacetate. The interrelationships among the acetylation products of protocevine, cevacine and cevadine are summarized in Chart II.

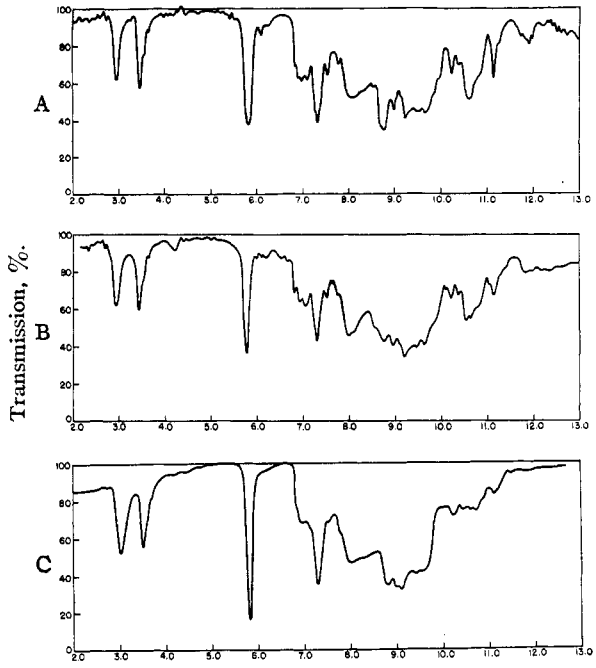
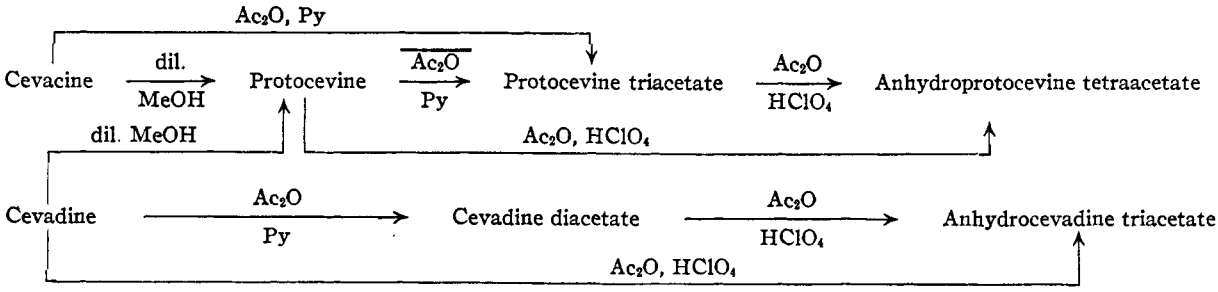


Fig. 2.—Infrared spectra in chloroform: A, cevadine diacetate; B, protocevine triacetate; C, cevagenine triacetate.

CHART II

ACETYLATION PRODUCTS OF PROTOCEVINE, CEVACINE AND CEVADINE



Comparison of the infrared spectra of anhydrocevadine triacetate, anhydroprotocevine tetraacetate and anhydrocevagenine triacetate lends further support to the conclusion that protocevine is the parent alkamine of cevadine (Fig. 3).

Comparison of the infrared spectrum of veratridine with that of the ester derivatives of protocevine, on the one hand, and of the esters of cevagenine on the other, indicates a far closer resemblance of veratridine to the protocevine esters. This evidence and the mildness of the conditions under which protocevine can be obtained from veratridine by alkaline hydrolysis, lead us to feel that protocevine is the parent alkamine of veratridine as well as of cevadine and cevacine.

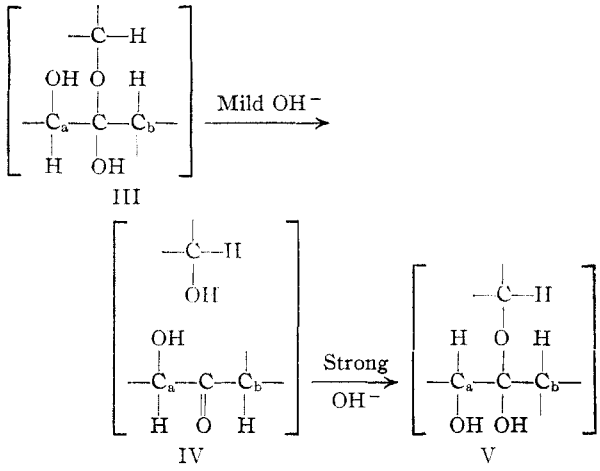
Barton and Eastham[12] have proposed an α-ketol hemiketal partial formulation for cevine. They have suggested that the isomerization of cevagenine to cevine involves a rearrangement of the α-ketol system. Those authors have also compared the relationship of the pairs germine–isogermine and protoverine–isoprotoverine to that of cevine–cevagenine.

We believe that protocevine can best be formulated as a labile α-ketol hemiketal system (III), which is opened easily and epimerized at C_b to give the α-ketol, cevagenine (IV). The isomerization of cevagenine to cevine would then involve epimerization at C_a and C_b followed by closure to form the stable α-ketol hemiketal (V).

(10) After the completion of this work, the paper by A. Stoll and E. Seebeck [*Helv. Chim. Acta*, **36**, 189 (1953)] described the isolation of γ-cevine from the alkaline hydrolysis mixture obtained from cevadine and veratridine. The close correspondence of the physical constants of the anhydro-acetates of protocevine and γ-cevine suggested the possible identity of the two materials. Dr. Stoll has kindly compared a sample of protocevine with γ-cevine and has reported to us that the two samples are identical.

(11) J. B. Niederl and V. Niederl, "Micromethods of Quantitative Organic Analysis," John Wiley and Sons, Inc., New York, N. Y., pp. 257–262. A three-hour hydrolysis period was used in this determination. This determination, and all other microanalyses reported, were carried out by Dr. S. M. Nagy and associates of the Massachusetts Institute of Technology. All samples were dried *in vacuo* at 120°.

(12) D. H. R. Barton and J. F. Eastham, *J. Chem. Soc.*, 424 (1953).



It appears likely that the alkamines zygadenine ($C_{27}H_{43}O_7N$), germine ($C_{27}H_{43}O_8N$) and protoverine ($C_{27}H_{43}O_9N$) all contain a labile α-ketol hemiketal system corresponding to that of protocevine, as evidenced by their facile conversion to carbonyl-containing isomers by mild alkaline treatment.[6,13] Hence the relation between germine and isogermine and between protoverine and isoprotoverine might better be compared to the relation between protocevine and cevagenine.

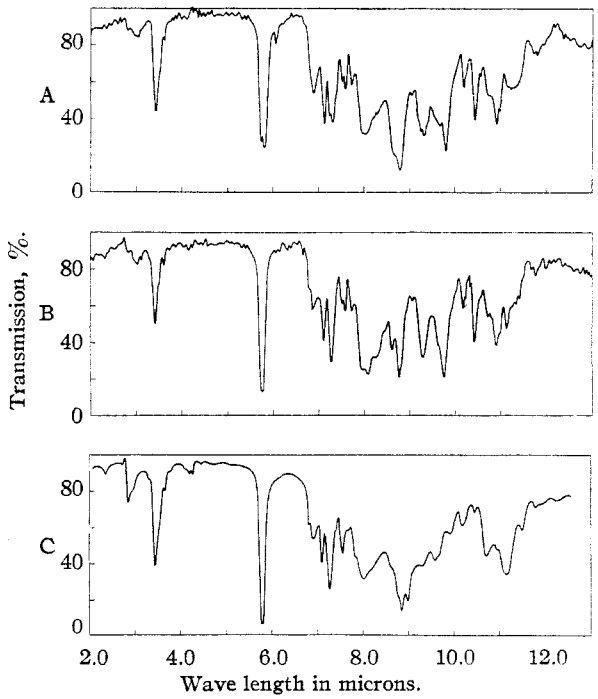


Fig. 3.—Infrared spectra in chloroform: A, anhydrocevadine triacetate; B, anhydroprotocevine tetraacetate; C, anhydrocevagenine triacetate.

Protocevine and cevacine were examined by Professor Otto Krayer for their circulatory and respiratory action in the anesthetized cat, their effect upon the failing heart of the heart-lung preparation of the dog, and their influence upon the amphibian skeletal muscle. In all three types of experiments the action of protocevine was similar to that of cevine. Cevacine qualitatively resembled veratridine in its respiratory, blood pressure and heart rate action, as well as in its action upon the failing heart, but was less potent. In causing the changes in muscular contraction characteristic of the veratrine response, cevacine was as potent as veratridine. The veratrinic action of cevacine, however, differed from the action of veratridine in that the relaxation after cevacine proceeded much faster than after veratridine.

(13) H. Jaffee and W. A. Jacobs, *J. Biol. Chem.*, **193**, 325 (1951).

Experimental

Fractionation of Veratrine.—To a solution of veratrine (100 g., S. B. Penick and Co., NF V)[14] in 1% sulfuric acid (1500 ml.) at 0–5°, 20% sodium nitrate solution was added dropwise until no further precipitation occurred (about 600 ml. of the nitrate solution was required). The nitrate salt was filtered and dried at 50°, yielding 85 g. The precipitate and filtrate were worked up as described in parts (a) and (b) below.

(a) The finely powdered nitrate salt (40 g.) was suspended in water (200 ml.) and cooled to 0–5°. The stirred suspension was made alkaline to *p*H 8.5 with 10% sodium hydroxide and then to *p*H 10 with aqueous ammonia. It was then extracted with ether (three 150-ml. portions) and chloroform (three 150-ml. portions). (Initial extraction with ether was found to be advisable to prevent troublesome emulsions during the chloroform extraction.) The ether and chloroform extracts were combined, washed with water and dried over sodium sulfate. Evaporation to dryness *in vacuo* left 36 g. of free base.

The free base obtained from the nitrate salt (36 g.) was dissolved in 5% sulfuric acid (160 ml.). A saturated solution of ammonium sulfate was added dropwise to permanent turbidity. Seeding with veratridine sulfate caused rapid separation of the sulfate salt on cooling. (In the absence of a seed, the sulfate separated slowly in the course of two days in the refrigerator.) After cooling overnight 11 g. of veratridine sulfate salt was collected by filtration. An additional 3 g. of sulfate separated from the filtrate on standing in the refrigerator for 3 days. The sulfate salt (14 g.) was recrystallized twice from water (using saturated ammonium sulfate to ensure complete precipitation). Regeneration of the free base with ammonia and extraction with ether gave 10 g. of veratridine, m.p. 160–180° dec., $[\alpha]^{25}D$ +6° (*c* 2.00, alc.).

The aqueous filtrate after the removal of veratridine sulfate was made alkaline to *p*H 10 with aqueous ammonia at 0–5° and extracted with ether (four 150-ml. portions). The combined ether extracts were washed with water and dried over sodium sulfate. Concentration of the ethereal solution to 30 ml. led to the separation of cevadine. After one day, 3.6 g., m.p. 203–207°, was obtained. When the ethereal solution was concentrated to dryness and the residue was crystallized from methanol–water, 5.0 g. of cevadine, m.p. 206–209°, was obtained. Both batches were combined and recrystallized from methanol–water, yielding 7.3 g. of cevadine, m.p. 209–211°, $[\alpha]^{25}D$ +11° (*c* 2.00, alc.).

(b) The aqueous filtrate after the removal of the nitrate salt was cooled to 0–5° and made alkaline with aqueous ammonia. The amorphous solid which separated (fraction A) was filtered and dissolved in ether, rejecting some inorganic salt which failed to dissolve. The ethereal solution was washed with water, dried over sodium sulfate and evaporated to dryness *in vacuo*, leaving 24 g. of amorphous solid. Crystallization of this material from methanol–water yielded 4.5 g. of cevadine, m.p. 208–210°, $[\alpha]^{25}D$ +10.5° (*c* 2.00, alc.).

The alkaline aqueous filtrate from the amorphous solid was extracted with ether (three 150-ml. portions). The ether extracts were combined, washed with water, dried over sodium sulfate, and evaporated to dryness, leaving 9 g. of amorphous solid (fraction B).

The alkaline aqueous solution was next extracted with chloroform (four 70-ml. portions) until the extract no longer gave a positive test with Mayer reagent. The com-

(14) We should like to thank S. B. Penick and Company for a generous gift of veratrine to Professor Otto Krayer, who kindly placed the material at our disposal.

bined chloroform extracts were washed with water and evaporated to dryness, leaving 1.5 g. of amorphous solid (fraction C).

Isolation of Cevacine (I) from Fraction B.—A solution of fraction B alkaloids (9 g.) in chloroform (50 ml., Merck reagent) was chromatographed on 200 g. of sulfuric acid-washed alumina[15] in a column of 30 mm. diameter. Elution with chloroform (200 ml.) and chloroform–2% methanol solution (400 ml.) gave alkaloidal fractions which crystallized from methanol–water to yield 3.5 g. of cevadine, m.p. 208–211°. Elution with chloroform–4% methanol (300 ml.) gave amorphous alkaloidal fractions whose infrared spectra indicated that they consisted of mixtures of cevadine and veratridine. The fractions eluted with chloroform–6% methanol (300 ml.) and chloroform–10% methanol (300 ml.) showed infrared absorption suggesting the presence of a new ester alkaloid. Crystallization of these fractions from acetone–water afforded glistening rectangular prisms (950 mg.), m.p. 205–207°, $[\alpha]^{21}D$ −27° (*c* 2.67, chf.).

Anal. Calcd. for $C_{27}H_{42}O_8N(COCH_3)$: C, 63.14; H, 8.22; N, 2.54; acetyl, 7.80. Found: C, 63.02; H, 8.52; N, 2.65; acetyl, 7.46.

Isolation of Protocevine (II) from Fraction C.—A solution of fraction C alkaloids (1.5 g.) in 20 ml. of chloroform was chromatographed on 30 g. of sulfuric acid-washed alumina in a column of 20 mm. diameter. Elution with chloroform (150 ml.) and chloroform–2% methanol (200 ml.) gave only traces of alkaloidal material. Elution with chloroform–4% methanol (200 ml.) gave an amorphous alkaloidal fraction from which cevacine (200 mg., m.p. 203–206°) was obtained by crystallization from acetone–water. Elution with chloroform–6% methanol (250 ml.) and chloroform–10% methanol (200 ml.) gave an amorphous fraction which crystallized from ether (220 mg.). Recrystallization of this material by solution in a large volume of boiling ether (100 ml.) and concentration to a small volume (20 ml.) yielded colorless needles (180 mg.). The product softened to a semi-solid mass at 179–183° and melted gradually up to 220–225° dec., $[\alpha]^{23}D$ −33° (*c* 2.13, chf.), $[\alpha]^{22}D$ −26° (*c* 1.80, alc.).

Anal. Calcd. for $C_{27}H_{43}O_8N$: C, 63.63; H, 8.51; N, 2.75. Found: C, 63.44; H, 8.65; N, 2.88.

Alkaline Isomerization of Protocevine to Cevagenine.—Protocevine (300 mg.) was added to a solution of 1 *N* NaOH (0.7 ml.) and methanol (4 ml.). The solution was heated under reflux for 15 minutes. It was then cooled and made just acid with 1:1 hydrochloric acid and the methanol was evaporated *in vacuo*. To the residue, water (2 ml.) and enough aqueous ammonia to bring the solution to *p*H 9 were added, and the base was extracted with chloroform (four 25-ml. portions). The chloroform solution was brought to dryness *in vacuo* and the residue was crystallized from chloroform–ether. Colorless needles (74 mg.) were obtained, which melted at 245–250° dec. after sintering at 170–180° and resolidifying at 190–195°. The melting point of the product was not depressed on admixture of a sample of cevagenine prepared from cevadine by the procedure of Stoll and Seebeck.[5] The infrared spectra of the two samples in Nujol were identical in all respects.

Alkaline Isomerization of Protocevine to Cevine.—Protocevine (300 mg.) was added to 20% alcoholic potassium hydroxide (2 ml.) and the solution was heated under reflux for 30 minutes. On cooling, long needles consisting of the potassium salt of cevine crystallized. The salt was filtered and dissolved in water (1 ml.). Addition of carbon dioxide caused the separation of a colorless solid. Recrystallization from dilute methanol yielded colorless prisms (90 mg.) which melted at 167–176°, $[\alpha]^{23}D$ −26° (*c* 2.16, chf.). The infrared spectrum in chloroform was identical with that of a sample of cevine prepared from cevadine.

Protocevine Triacetate.—A mixture of protocevine (200 mg.), acetic anhydride (4 ml.) and pyridine (2 ml.) was heated on the steam-bath for two hours. Evaporation of the solution to dryness *in vacuo* at room temperature left an amorphous solid, which was dissolved in water (3 ml.). The solution was made alkaline with aqueous ammonia and extracted with chloroform (three 35-ml. portions). The chloroform extract was washed with water (5 ml.) and evaporated to dryness *in vacuo*. The residue crystallized from ether–petroleum ether, and recrystallization of the product from the same solvents yielded protocevine triacetate (120 mg.) in the form of prisms, m.p. 239–241° dec., $[\alpha]^{22}D$ −22° (*c* 1.63, chf.).

Anal. Calcd. for $C_{27}H_{40}O_8N(COCH_3)_3$: C, 62.35; H, 7.76; acetyl, 20.31. Found: C, 62.59; H, 8.03; acetyl, 19.97.

Anhydroprotocevine Tetraacetate. A. From Protocevine.—To an ice-cooled suspension of protocevine (1 g.) in acetic anhydride (6 ml.), 70% perchloric acid (0.2 ml., Merck Reagent) was added dropwise with stirring, and the brown solution was allowed to stand overnight at room temperature. Methanol (9 ml.) was then added dropwise carefully and, after one hour, the solution was brought to dryness *in vacuo*. The residue crystallized from acetone–water, and recrystallization from the same solvents yielded anhydroprotocevine tetraacetate perchlorate (625 mg.) in the form of long needles, m.p. 253–254° dec.

Anal. Calcd. for $C_{35}H_{49}O_{11}N\cdot HClO_4$: C, 55.29; H, 6.50. Found: C, 55.06; H, 6.84.

The perchlorate salt (300 mg.) was treated with 2 *N* aqueous ammonia, and the liberated base was extracted with benzene (three 25-ml. portions). Evaporation of the benzene solution to dryness *in vacuo* left an amorphous residue (220 mg.), which crystallized from acetone–water. Recrystallization from the same solvents yielded anhydroprotocevine tetraacetate (140 mg.) in the form of colorless needles, m.p. 253–255° dec., $[\alpha]^{23}D$ +77° (*c* 1.90, alc.).

Anal. Calcd. for $C_{35}H_{49}O_{11}N$: C, 63.71; H, 7.48; acetyl, 26.09. Found: C, 64.08; H, 7.65; acetyl, 27.32.

B. From Protocevine Triacetate.—Protocevine triacetate (250 mg.) was treated with acetic anhydride (1.6 ml.) and 70% perchloric acid (0.08 ml.) and the reaction mixture was worked up as described above in part A. Crystallization of the crude residue from acetone–water gave anhydroprotocevine tetraacetate perchlorate (120 mg.), m.p. 252–254°. Liberation of the free base with dilute aqueous ammonia as above gave anhydroprotocevine tetraacetate (60 mg.), m.p. 252–253° dec. The melting point of the product was not depressed on admixture of anhydroprotocevine tetraacetate prepared from protocevine, and the infrared spectra of the two samples were identical.

Methanolysis of Cevacine to Protocevine.—Cevacine (300 mg.) was added to a solution of methanol (10 ml.) and water (5 ml.) and the solution was allowed to stand at room temperature for 15 hours. The methanol and water were evaporated *in vacuo* and chloroform was added and boiled off to remove the last traces of water. The amorphous residue was boiled with ether (40 ml.) and filtered from insoluble solid. After concentration of the filtrate to about 10 ml., colorless needles (140 mg.) separated. The product formed a semi-solid mass at 179–183° and melted gradually up to 220–225° dec. The infrared spectrum of this material in chloroform was identical with that of protocevine.

Acetylation of Cevacine to Protocevine Triacetate.—Cevacine (300 mg.) was acetylated with acetic anhydride (6 ml.) and pyridine (3 ml.) as described above for the acetylation of protocevine. Crystallization of the crude amorphous product from ether–petroleum ether gave clusters of prisms (181 mg.), m.p. 239–241° dec. The melting point of this product was not depressed on admixture of protocevine triacetate and its infrared spectrum was identical with that of protocevine triacetate.

Alkaline Hydrolysis of Cevacine to Cevagenine.—Cevacine (300 mg.) was treated with aqueous methanolic sodium hydroxide and the hydrolysis mixture was worked up as described above for the isomerization of protocevine to cevagenine. The crystalline product from chloroform–ether (80 mg.) melted at 245–250° dec. after sintering at 170–180° and resolidifying at 190–195°. The melting point of the product was not depressed on admixture of cevagenine, and the infrared spectrum of the material in Nujol was the same as that of cevagenine.

Alkaline Hydrolysis of Cevacine to Cevine.—Cevacine (300 mg.) was treated with 20% alcoholic potassium hydroxide and the hydrolysis mixture was worked up as described above for the isomerization of protocevine to cevine. The crystalline product from methanol–water (84 mg.) melted at 167–176°. The mixed melting point with an authentic sample of cevine was not depressed, and the in-

(15) We wish to thank Dr. Max Tishler of Merck and Company for generously supplying us with Merck sulfuric acid-washed alumina.

frared spectra of the two samples in chloroform were identical.

Methanolysis of Cevadine to Protocevine. A. At Room Temperature.—Cevadine (500 mg.) was dissolved in a mixture of methanol (15 ml.) and water (5 ml.) and the solution was allowed to stand for five days at room temperature. The solvents were then concentrated until the solution became turbid. Seeding with cevadine led to the separation of crystalline starting material (440 mg.), m.p. 210–212°. The filtrate was evaporated to dryness *in vacuo* and benzene was added and boiled off to remove the last traces of water. The amorphous residue was boiled with ether (30 ml.) and filtered from insoluble solid. Concentration of the filtrate led to the separation of protocevine (30 mg.), m.p. 220–225° dec. after sintering from 175°.

B. **At Reflux Temperature.**—Cevadine (2 g.) was dissolved in a mixture of methanol (10 ml.) and water (5 ml.) and the solution was heated under reflux for five days. Concentration of the solvents and seeding with cevadine led to the recovery of 390 mg. of unchanged starting material. The filtrate was evaporated to dryness *in vacuo* and dried by adding and boiling off benzene. The amorphous residue crystallized from chloroform–ether, yielding cevagenine (75 mg.), m.p. 245–248° dec. after sintering from 170°. Evaporation of the filtrate to dryness left a residue which crystallized from ether yielding protocevine (260 mg.), m.p. 220–225° dec. after sintering from 170°; infrared spectrum in chloroform identical with that of the analytical sample above.

Alkaline Hydrolysis of Cevadine to Protocevine.—To a solution of cevadine (5.92 g., 0.01 mole) in methanol (150 ml.) and water (60 ml.) was added 4% sodium hydroxide solution (10.1 ml., 0.011 mole) and the solution was heated under reflux for 10 minutes. The slightly yellow solution was cooled and made just acid with 1:1 hydrochloric acid and the methanol was evaporated *in vacuo*. To the residue, cooled to 0–5°, was added 10% sodium hydroxide solution to neutrality and then aqueous ammonia to *p*H 9. The alkaline suspension was extracted with chloroform (four 50-ml. portions) and the chloroform solution was washed and concentrated to 30 ml. Addition of ether (20 ml.) led to separation of cevagenine (1.0 g., m.p. 245–248° dec. after sintering from 170°). Evaporation of the filtrate to dryness left a residue which crystallized from ether (1.68 g.). Recrystallization from ether gave protocevine (1.39 g.), m.p. 220–225° after sintering from 175°; infrared spectrum in chloroform identical with that of the analytical sample above.

Alkaline Hydrolysis of Veratridine to Protocevine.—Veratridine (6.73 g., 0.01 mole) was treated with dilute methanolic sodium hydroxide and the reaction mixture worked up as described above for the alkaline hydrolysis of cevadine. From the chloroform–ether solution, cevagenine (0.81 g.) was obtained. From the residue after removal of cevagenine, 1.9 g. of crude crystalline product was obtained from ether. Recrystallization from the same solvent yielded protocevine (1.53 g.), m.p. 220–225° dec. after sintering from 179°; infrared spectrum identical in chloroform with that of the analytical sample above.

Anhydrocevadine Triacetate.—Cevadine was acetylated with acetic anhydride and perchloric acid according to the procedure described by Stoll and Seebeck.[8] Recrystallization of the product from ether gave rectangular plates, m.p. 278–280° dec., $[\alpha]_D$ +90° (*c* 2.15, chf.).

Anal. Calcd. for $C_{35}H_{53}O_{11}N$: C, 65.22; H, 7.63. Found: C, 65.64; H, 7.80.

In a volatile acid determination[11] 35.96 mg. of substance yielded an amount of acid equivalent to 24.45 ml. of 0.008103 *N* sodium thiosulfate; calcd. for anhydroprotocevine triacetate monoangelate, 25.37 ml.

Cevadine Diacetate.—Cevadine was acetylated with acetic anhydride and pyridine according to the procedure of Stoll and Seebeck.[8] Recrystallization of the crude product from ether–petroleum ether yielded colorless needles, m.p. 258–260° dec., $[\alpha]^{23}D$ −13° (*c* 2.17, alc.).

Cevagenine Triacetate.—Cevagenine (3.0 g.) was acetylated with acetic anhydride and pyridine at room temperature according to the procedure of Stoll and Seebeck.[5] Crystallization of the crude product from ether afforded anhydrocevagenine triacetate (prisms, 1.08 g., m.p. 285–286° dec.). Concentration of the filtrate led to the separation of cevagenine triacetate in the form of needles (0.93 g.). Repeated crystallization from acetone–ether gave needles (0.45 g.) which melted at 242–243.5° dec., $[\alpha]^{22}D$ −62° (*c* 1.33, alc.).

Anhydrocevagenine Triacetate.—Cevagenine was treated with acetic anhydride and pyridine at steam-bath temperature according to the directions of Stoll and Seebeck.[8] The product melted at 285–287° dec., $[\alpha]^{23}D$ −47° (*c* 1.17, alc.).

CAMBRIDGE, MASSACHUSETTS

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Isolation of Amino Acids by Distillation of the Acetylated Amino Acid Ethyl Esters

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Mixtures of acetylated amino acid ethyl esters were prepared from protein hydrolyzates, and the mixed esters were fractionated in efficient distilling columns. The esters are sufficiently stable to withstand efficient fractionation procedures. Fractions rich in alanine, valine, leucine, isoleucine, proline, aspartic acid, glutamic acid, methionine and phenylalanine were obtained under moderate fractionation. By more precise fractionation, alanine, valine, leucine and isoleucine were obtained as the pure amino acids. These pure amino acids retained an appreciable percentage of their original optical activity.

Introduction

The slow development of a commercial amino acid industry as compared with the rapid development of the fatty acid industry is probably due in large part to the difficulty and expense involved in isolating or synthesizing the amino acids in quantity. General technological advances in recent years have indicated that further knowledge of the separation of the volatile derivatives of the amino acids may lead to an economical means of producing the amino acids from proteinaceous agricultural products.

The work of Fischer[2] on the distillation of the amino acid ethyl esters aroused considerable interest for a time because it appeared to offer an excellent means of separating the amino acids from a protein hydrolyzate. A wide variety of proteins were subjected to analysis by this method, but the results were far from satisfactory. Each of the fractions obtained contained a mixture of all the amino acids present, and a considerable residue—apparently amino acid anhydrides—remained in the still pot.

Morgan[3] prepared the butyl esters of some of the amino acids and claimed that they were consider-

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(2) E. Fischer, *Ber.*, **34**, 433 (1901).

(3) W. T. J. Morgan, *J. Chem. Soc.*, 79 (1926).