A Synthesis of [1-(N-Methyl-hemi-L-cystine)]-oxytocin and a Study of Its Reaction with Acetone[1]

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Abstract: [1-(N-Methyl-hemi-L-cystine)]-oxytocin, an analog of the posterior pituitary hormone oxytocin in which one of the hydrogens of the free amino group is replaced by a methyl group, has been synthesized and tested for biological activity. Preparations of this analog (N-methyl-oxytocin) were found to differ markedly in biological potency and it was suspected that this variation might be due to the presence of varying amounts of oxytocin as a trace contaminant. The N-methyl-S-benzylcysteine used in the synthetic procedures may have contained undetectable traces of S-benzylcysteine which, if present, could account for the eventual formation of trace amounts of oxytocin. For removal of any oxytocin present, an N-methyl-oxytocin preparation was treated with acetone, since it was found that N-methyl-oxytocin reacts with acetone to only a slight extent under conditions (80% aqueous acetone, 22 hr) which convert oxytocin almost quantitatively to "acetone-oxytocin." Small amounts of "acetone-oxytocin" and "acetone-N-methyl-oxytocin" were formed and were readily separated from unreacted N-methyl-oxytocin by partition chromatography on Sephadex G-25. N-Methyl-oxytocin purified in this manner was found to possess approximately 2 units/mg of oxytocic activity, compared with 500 units/mg for oxytocin. "Acetone-N-methyl-oxytocin" was formed in higher yield by treatment of the N-methyl-oxytocin with 80% aqueous acetone for 7 days and was isolated and purified by partition chromatography and gel filtration on Sephadex G-25.

In the course of earlier studies on the importance of the chemical functional groups of the posterior pituitary hormone oxytocin to its biological activities, the synthesis of [1-(N-methyl-hemi-L-cystine)]-oxytocin was undertaken. In this analog, which will be referred to as N-methyl-oxytocin, the free amino group on the half-cystine residue at position 1 in oxytocin (Figure 1) is replaced by a methylamino group. The required protected nonapeptide intermediate for the synthesis of N-methyl-oxytocin, namely N-carbobenzoxy-N-methyl-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, was obtained by the stepwise *p*-nitrophenyl ester method employed by Bodanszky and du Vigneaud for the synthesis of oxytocin.[3] The protected nonapeptide was treated with sodium in liquid ammonia to remove the N-carbobenzoxy and S-benzyl protecting groups by the method of Sifferd and du Vigneaud[4] as used in the original synthesis of oxytocin.[5] Oxidation of the resulting disulfhydryl compound and isolation of the N-methyl-oxytocin by countercurrent distribution[6] gave a preparation possessing a potency of approximately 3 units/mg in the avian vasodepressor assay.[7] Oxytocin exhibits approximately 500 units/mg of this activity.[8] Repetition of the synthesis of N-methyl-oxytocin gave another preparation with a potency of approximately 6 units/mg. The possibility was considered that different amounts of oxytocin might be present as a trace contaminant in the two preparations of N-methyl-oxytocin. The traces of oxytocin could be accounted for by the presence of small amounts of unmethylated S-benzyl-L-cysteine in the N-methyl-S-benzyl-L-cysteine used in the synthesis of the protected nonapeptide.

Jošt, Rudinger, and Šorm have synthesized N-methyl-oxytocin by a different route.[9] Their preparation possessed an avian vasodepressor potency of about 0.025 unit/mg and an oxytocic potency (rate uterus *in vitro*) of about 0.25 unit/mg. Since an earlier preparation reported by Rudinger[10] possessed higher avian vasodepressor and oxytocic activities, 0.95 and 1.5 units/mg, respectively, they discussed in some detail the possibility of contamination of N-methyl-oxytocin with oxytocin. However, because their N-methyl-oxytocin preparation of lower activity possessed ratios of one activity to another which deviated considerably from the corresponding ratios for oxytocin, they concluded that the activity found for the N-methyl-oxytocin is a genuine property of the analog.

Repeated attempts on our part to demonstrate conclusively the presence of oxytocin in our preparations of N-methyl-oxytocin and to separate oxytocin from the analog were unsuccessful. On countercurrent distribution and on partition chromatography on Sephadex G-25, N-methyl-oxytocin and oxytocin had almost the same K and R_f values in the solvent systems employed. Furthermore, separation of oxytocin from a mixture of oxytocin and the N-methyl analog was not effected by the use of paper electrophoresis or paper chromatography.

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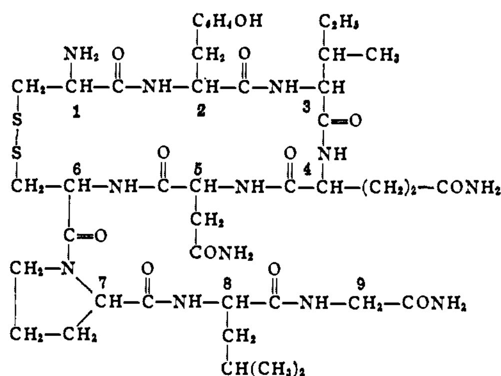

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues.

Recently we have found in connection with studies on the nature of the inactivation of oxytocin by acetone[11] that N-methyl-oxytocin reacts to only a slight extent with acetone under conditions (80% aqueous acetone, 22 hr) which convert oxytocin almost quantitatively to the derivative "acetone-oxytocin." It occurred to us that upon treatment of an N-methyl-oxytocin preparation with acetone under these conditions, any traces of oxytocin should react selectively with the acetone to give "acetone-oxytocin" which might then be separable from the analog.

When a sample of N-methyl-oxytocin, prepared as described in the Experimental Section, was treated with 80% aqueous acetone for 22 hr, the analog was recovered in good yield by partition chromatography on Sephadex G-25[12] in 1-butanol–benzene–pyridine–0.1% aqueous acetic acid (6:2:1:9) (solvent system A). N-Methyl-oxytocin was detected as a large peak with R_f 0.27, very close to the known position of oxytocin (R_f 0.24).[12] A very small fast-moving peak with R_f 0.82 was also observed.

When the small amount of material isolated from the fast-moving peak was subjected to partition chromatography in 1-butanol–benzene–pyridine–0.1% aqueous acetic acid (7:9:2:18) (solvent system B), a peak with R_f 0.25, the same R_f as that of "acetone-oxytocin," was obtained. Another peak appeared at R_f 0.51. The material in this latter peak, obtained here in an amount representing only a small portion of the original sample of N-methyl-oxytocin subjected to acetone treatment, was later obtained under different conditions of acetone treatment in much higher yield and identified as an acetone derivative of N-methyl-oxytocin, as will be discussed subsequently. The material represented by the peak at R_f 0.25 was treated under conditions known to regenerate oxytocin from "acetone-oxytocin": heating in 0.25% acetic acid at 90° for 30 min. The solution was lyophilized and the resulting material was subjected to partition chromatography in solvent system A. The presence of oxytocin was established by its R_f value and by bioassay of the isolated material.

The N-methyl-oxytocin preparation isolated after acetone treatment was assayed for biological activity. The assay for oxytocic activity[13] was used because the assay for avian vasodepressor activity[7] did not discriminate satisfactorily between the various dose levels of this highly purified N-methyl-oxytocin. The oxytocic potency of the analog was approximately 2 units/mg after acetone treatment as compared to about 5 units/mg for the analog preparation before acetone treatment. Oxytocin exhibits approximately 500 units/mg of oxytocic activity.[8] Treatment of the N-methyl-oxytocin a second time with acetone caused no further change in oxytocic potency, indicating that the oxytocic activity found after the first treatment with acetone was that of the analog.

When the original N-methyl-oxytocin preparation was treated with 80% aqueous acetone for 7 days and then subjected to partition chromatography on Sephadex G-25 in solvent system A, the chromatogram showed the presence of the same compounds as those obtained in the original 22-hr treatment of N-methyl-oxytocin with acetone. The fast-moving peak (R_f 0.76) was now larger than the slow-moving peak (R_f 0.25) corresponding to N-methyl-oxytocin. More than twice as much material was isolated from the fast-moving peak as from the N-methyl-oxytocin peak. Subjection of the material of R_f 0.76 to partition chromatography in solvent system B gave a major peak with R_f 0.53. The material isolated from this peak was further purified, first by partition chromatography again in solvent system B, and then by gel filtration on Sephadex G-25 in 0.2 *N* acetic acid.

The highly purified material liberated acetone when it was heated with 2,4-dinitrophenylhydrazine in dilute methanolic HCl, as indicated by the formation of acetone 2,4-dinitrophenylhydrazone. Elementalan alyses of the acetone–peptide compound agreed with values calculated for a monoisopropylidene derivative of N-methyl-oxytocin. When a sample of this derivative, "acetone-N-methyl-oxytocin," was analyzed for amino acids and ammonia,[14] the expected constituents were found in the same molar ratios as for N-methyl-oxytocin. On paper electrophoresis at pH 5.6 this derivative of N-methyl-oxytocin migrated toward the cathode at a rate considerably slower than that of N-methyl-oxytocin.

In the studies on "acetone-oxytocin" it was found that acetone is liberated from the compound, mole for mole, when the "acetone-oxytocin" is boiled in 0.1 *N* acetic acid for 10 min.[11] When the "acetone-N-methyl-oxytocin" was boiled in 0.1 *N* acetic acid for 6 min, about 25% of the theoretical amount of acetone was liberated. Since it appeared that the "acetone-N-methyl-oxytocin" liberates acetone more slowly than does "acetone-oxytocin," the compound was heated with 0.1 *N* acetic acid in a sealed tube at 95° for 20 hr before the quantitative determination of acetone was carried out. Under these conditions the "acetone-N-methyl-oxytocin" liberated 98% of the amount of acetone theoretically obtainable from a monoisopropylidene derivative of N-methyl-oxytocin.

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Experimental Section[15]

Methods. Sephadex G-25 columns for partition chromatography were prepared and operated as described previously.[12] Gel filtration was performed on Sephadex G-25 block polymerizate (200–270 mesh, U. S. Standard sieve series). The solvent system 1-butanol–benzene–pyridine–0.1% aqueous acetic acid (6:2:1:9) is referred to as solvent system A and 1-butanol–benzene–pyridine–0.1% aqueous acetic acid (7:9:2:18) as solvent system B. Peptide materials in the Sephadex column effluents were detected by the Folin–Lowry procedure.[16] The method of Ellman was used for the determination of sulfhydryl groups.[17]

N-Methyl-S-benzyl-L-cysteine. L-Thiazolidine-4-carboxylic acid[18] (14.8 g) was dissolved in liquid ammonia (600 ml) containing water (2.2 ml). Sodium was added in small pieces until a blue color persisted throughout the solution for 15 min.[19] The blue color was discharged with NH_4Cl and benzyl chloride (14 ml) was added. After the mixture was stirred for 2 hr, the ammonia was allowed to evaporate, and the resulting residue was dissolved in water (200 ml). The mixture was washed with ether (50 ml) and filtered. The pH of the filtrate was adjusted to 6 with concentrated HCl followed by glacial acetic acid. The solid was collected on a filter, washed with ice-cold water, and dissolved in 1 *N* HCl (400 ml). Insoluble material was filtered off, and the pH of the filtrate was adjusted to 6 with 10 *N* NaOH and glacial acetic acid. The precipitated N-methyl-S-benzyl-L-cysteine was collected on a filter and washed with ice-cold water, alcohol, and ether: yield 22.5 g, mp 203–204° dec. A sample recrystallized from hot water had mp 205–206° dec and $[\alpha]^{20}D$ +67.5° (*c* 1, 1 *N* HCl) (lit.[20] mp 207–208° dec, $[\alpha]D$ +64.5° (*c* 1, 1 *N* HCl)).

N-Carbobenzoxy-N-methyl-S-benzyl-L-cysteine. N-Methyl-S-benzyl-L-cysteine (2.25 g) was dissolved in 1 *N* NaOH (30 ml), and the solution was cooled to 0°. Carbobenzoxy chloride (2.56 g) was added, and the mixture was stirred for 2 hr without further cooling while the pH was maintained at 8 by addition of 1 *N* NaOH. The mixture was washed with ether (50 ml) and then made acid to congo red with 6 *N* HCl. The product was extracted with three 50-ml portions of ethyl acetate. The combined ethyl acetate extracts were washed with 10% NH_4Cl and dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the residue was crystallized from ethyl acetate–hexane to give 3.0 g, mp 72–73°, $[\alpha]^{20}D$ −109.5° (c 1, absolute ethanol).

Anal. Calcd for $C_{19}H_{21}NO_4S$: C, 63.5; H, 5.89; N, 3.90. Found: C, 63.6; H, 5.90; N, 3.90.

***p*-Nitrophenyl N-Carbobenzoxy-N-methyl-S-benzyl-L-cysteinate.** N-Carbobenzoxy-N-methyl-S-benzyl-L-cysteine (2.17 g) and *p*-nitrophenol (0.84 g) were dissolved in ethyl acetate (40 ml). The solution was cooled in an ice bath and stirred while dicyclohexylcarbodiimide (1.27 g) was added. Stirring was continued for 1 hr at 0° and then for 4 hr at room temperature. Glacial acetic acid (0.1 ml) was added, and the mixture was stirred for an additional 2 hr. The N,N′-dicyclohexylurea which had separated was filtered off and washed with ethyl acetate (15 ml). The combined filtrate and washing were evaporated *in vacuo*, and the residue was dissolved in chloroform (5 ml) and passed through a silica gel column (2.8 × 13 cm) with chloroform as eluent. That portion of the eluate containing the product, as detected by its uv fluorescence, was evaporated *in vacuo* to dryness to give 2.65 g of the ester, $[\alpha]^{24}D$ −89° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{25}H_{24}N_2O_6S$: C, 62.5; H, 5.03; N, 5.83. Found: C, 62.6; H, 5.14; N, 5.90.

N-Carbobenzoxy-N-methyl-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[3] was treated with hydrogen bromide in trifluoroethanol and the resulting hydrobromide salt was treated with liquid ammonia as described previously.[12b] The free octapeptide so obtained (1.25 g) was dissolved in a mixture of dimethylformamide (10 ml), water (1 ml), and glacial acetic acid (0.072 ml). *p*-Nitrophenyl N-carbobenzoxy-N-methyl-S-benzyl-L-cysteinate (0.66 g) was added, and after 24 hr the mixture was poured into ethyl acetate (90 ml). The product was collected on a filter and washed with ethyl acetate (50 ml), absolute ethanol (20 ml), and absolute ethanol–ethyl acetate (1:1) (40 ml) to give 1.26 g, mp 209–213° dec, $[\alpha]^{22}D$ −57° (*c* 1, dimethylformamide). This product was used in the next step. For analysis a sample (200 mg) was dissolved in 3.2 ml of glacial acetic acid and precipitated by slow addition of 8.7 ml of water to give 142 mg, mp 216–219° dec, $[\alpha]^{22}D$ −65° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{66}H_{88}N_{12}O_{14}S_2$: C, 59.3; H, 6.63; N, 12.6. Found: C, 59.1; H, 6.59; N, 12.3.

N-Methyl-oxytocin. The protected nonapeptide (460 mg), prepared as described in the preceding section, was dissolved in 150 ml of liquid ammonia (distilled from sodium) and treated at the boiling point of the solution with sodium until a blue color spread throughout the entire solution. Approximately 60 mg of sodium was required. The solution was evaporated *in vacuo* to a volume of about 20 ml and dried in the frozen state. The product was dissolved in deaerated water (500 ml) containing trifluoroacetic acid (0.18 ml). The pH of the solution was about 8 and the sulfhydryl content was 0.600 mmol. The solution was titrated with 0.1 *N* potassium ferricyanide[21] (6.1 ml) while the pH was maintained near 7 with 1 *N* NH_4OH. When the yellow color of excess oxidant persisted in the solution, the sulfhydryl content was 0.003 mmol. The solution was stirred with the trifluoroacetate form of Bio-Rad AG3X4 resin (15 ml wet volume) for 15 min. The resin was filtered off and the solution was lyophilized. The crude product was dissolved in 12 ml of the organic phase of solvent system A and subjected to partition chromatography in solvent system A on a Sephadex column (2.82 × 114.7 cm). In the resulting chromatogram N-methyl-oxytocin appeared as a large peak with R_f 0.27 and isolation of the material represented by this peak gave 132 mg of the analog possessing an oxytocic activity on the isolated rat uterus[13] of about 4.6 units/mg. A sample (127 mg) of N-methyl-ocytocin was dissolved in 80% (v/v) aqueous acetone (127 ml) and allowed to stand at room temperature for 22 hr. Water (25 ml) was added, the acetone was removed *in vacuo*, and the resulting aqueous solution was lyophilized. The product was dissolved in 2.5 ml of the organic phase of solvent system A and subjected to partition chromatography in solvent system A on a Sephadex column (2.83 × 55 cm). The resulting chromatogram showed two peaks with R_f values of 0.82 and 0.27, the latter corresponding to N-methyl-oxytocin. Isolation of the materials represented by these peaks gave 10.1 mg of the fast-moving substance (R_f 0.82) and 93.3 mg of N-methyl-oxytocin possessing an oxytocic potency of 1.8 units/mg. A sample (91.5 mg) of this N-methyl-oxytocin was treated with 80% aqueous acetone a second time in the same manner and then was subjected again to partition chromatography under the same conditions. The chromatogram showed the presence of two peaks with R_f values of 0.75 and 0.26, and isolation of the materials represented by these peaks gave 6.9 mg of the fast-moving substance (R_f 0.75) and 63.5 mg of N-methyl-oxytocin. When this preparation of N-methyl-oxytocin was assayed for oxytocic activity against the preparation that had been treated only once with acetone, the two samples were found to possess the same oxytocic potency (1.8 units/mg).

A sample (81.6 mg) of N-methyl-oxytocin that had been treated once with acetone and isolated by partition chromatography was subjected to gel filtration on a Sephadex G-25 column (2.82 × 62.2 cm) in 0.2 *N* acetic acid. In the resulting chromatogram the N-methyl-oxytocin appeared as a peak with a maximum at effluent volume 290 ml, and isolation of the material gave 64.9 mg of the analog, $[\alpha]^{21}D$ −52° (*c* 0.5, 1 *N* acetic acid). For analysis a sample was dried at 100° over phosphorus pentoxide *in vacuo* with a loss in weight of 4.2%.

Anal. Calcd for $C_{44}H_{68}N_{12}O_{12}S_2$: C, 51.8; H, 6.71; N, 16.5. Found: C, 51.6; H, 6.77; N, 16.3.

A sample was hydrolyzed in 6 *N* HCl *in vacuo* at 110° for 48 hr and analyzed[14] in a Beckman-Spinco amino acid analyzer. The following molar ratios of amino acids and ammonia were found, with the value of glycine taken as unity: aspartic acid, 1.1; glu-

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tamic acid, 1.1; proline, 1.0; glycine, 1.0; cystine, 0.27; mixed disulfide of cysteine and N-methylcysteine, 0.34; isoleucine, 1.0; leucine, 1.0; tyrosine, 0.9; and ammonia, 3.1. The values for cystine and the mixed disulfide account for the half-cystine residue in this analog.[22]

"Acetone-N-methyl-oxytocin." A sample (138 mg) of N-methyl-oxytocin that had not been treated with acetone was dissolved in 80% aqueous acetone (138 ml) and allowed to stand at room temperature in the dark for 7 days. The product isolated by evaporation and lyophilization was dissolved in 5 ml of the organic phase of solvent system A and subjected to partition chromatography in solvent system A on a Sephadex column (2.83 × 53.9 cm). The resulting chromatogram showed two peaks with R_f values of 0.76 and 0.25, the latter corresponding to N-methyl-oxytocin. Isolation of the materials represented by the peaks gave 84.3 mg of the fast-moving substance (R_f 0.76) and 37.2 mg of N-methyl-oxytocin. The material from the fast-moving peak was dissolved in 8 ml of the organic phase of solvent system B and subjected to partition chromatography in solvent system B on a Sephadex column (2.83 × 53.8 cm). The resulting chromatogram showed two peaks with R_f values of 0.82 and 0.53 with the latter being the major peak. The material represented by the major peak (61.4 mg) was dissolved in 4 ml of the organic phase of solvent system B and rechromatographed in solvent system B. The resulting chromatogram showed one peak with R_f 0.53 with a slight shoulder on the leading edge. Isolation of material corresponding to the central portion of the peak gave 42.7 mg. For further purification a sample (31.3 mg) of this material was dissolved in 0.2 *N* acetic acid (5 ml) and subjected to gel filtration on a Sephadex G-25 column (2.82 × 62.2 cm) in 0.2 *N* acetic acid. Isolation of material corresponding to the central portion of the major peak (maximum at effluent volume 343 ml) in the resulting chromatogram gave 24.1 mg of highly purified acetone-N-methyl-oxytocin, $[\alpha]^{21}D$ −136° (*c* 0.5, 1 *N* acetic acid). For analysis a sample was dried at 100° over phosphorus pentoxide *in vacuo* with a loss in weight of 4.0%.

Anal. Calcd for $C_{47}H_{72}N_{12}O_{12}S_2$: C, 53.2; H, 6.84; N, 15.8. Found: C, 53.1, H, 6.93; N, 15.6.

A sample was hydrolyzed in 6 *N* HCl *in vacuo* at 110° for 48 hr and analyzed in a Beckman-Spinco amino acid analyzer. The following molar ratios of amino acids and ammonia were found, with the value of glycine taken as unity: aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.1; glycine, 1.0; cystine, 0.28; mixed disulfide of cysteine and N-methylcysteine, 0.36; leucine, 1.0; isoleucine, 1.1; tyrosine, 0.9; and ammonia, 3.0. These values are the same, within the experimental error, as those found for N-methyl-oxytocin.[22]

As in previous studies with "acetone-oxytocin,"[11] the liberation of acetone from the isopropylidene derivative of N-methyl-oxytocin was demonstrated by heating a sample with 2,4-dinitrophenylhydrazine in dilute methanolic HCl at 90° for 20 min and identification by paper chromatography of the acetone 2,4-dinitrophenylhydrazone so obtained. The quantitative determination of acetone liberated by the derivative was also carried out according to the procedure described previously.[11] When a sample (1.017 mg) was boiled in 0.1 *N* acetic acid (5 ml) for 6 min, the amount of acetone liberated was 25% of that theoretically obtainable from a monoisopropylidene derivative of N-methyl-oxytocin. When a sample (0.857 mg) was heated in 0.1 *N* acetic acid (1.2 ml) in a sealed tube at 95° for 20 hr, the amount of acetone liberated was 98% of the theoretical value.

On paper electrophoresis in pyridine acetate buffer of pH 5.6 (18 hr at 4° and 300 V) the isopropylidene derivative of N-methyl-oxytocin migrated toward the cathode at a considerably slower rate than N-methyl-oxytocin, as detected by color development with the Pauly reagent.

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(22) The position and color value for the mixed disulfide of cysteine and N-methylcysteine was obtained by analysis of an equimolar mixture of L-cystine and N,N′-dimethyl-L-cystine (see ref 20) after the mixture had been heated in 6 *N* HCl *in vacuo* at 110° for 48 hr. In the analysis of N-methyl-oxytocin the mixed disulfide appeared just ahead of the glutamic acid peak and a small shoulder containing N,N′-dimethyl-L-cystine appeared on the leading edge of the aspartic acid peak.

Biosynthesis of Indole Alkaloids. Vindoline[1]

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Abstract: The biosynthesis of vindoline in *Vinca rosea* plants has been investigated using appropriate radioactive precursors. The results obtained are in full accord with the suggested monoterpenoid origin for the nontryptophan-derived portion of the indole alkaloids.

In spite of the structural diversity shown by indole alkaloids[2,3] it has become increasingly obvious that most of them can be conveniently classified into three main structural groups.[4] These are described as the corynanthe, aspidosperma, and iboga groups and may be structurally derived (Figure 1) by combination of a tryptamine residue with C_{10} units (**1**, **2**, and **3**, respectively). Ajmaline (**4**), vindoline (**5**), and catharanthine (**6**) are shown as representative examples of each of the main groups. Structural variations within each group are numerous, but in spite of this the basic patterns are discernible. Certain alkaloids contain a C_9 unit in addition to the tryptamine residue, and it is invariably the carbon atom indicated by the dotted lines in **1**, **2**, and **3** which is lost.

The reality of this structural classification (Figure 1) in biosynthetic terms has been extensively probed, and

(1) Much of this work has been described briefly in communications: (a) T. Money, I. G. Wright, F. McCapra, and A. I. Scott, *Proc. Natl. Acad. Sci. U. S.*, **53**, 901 (1965); (b) F. McCapra, T. Money, A. I. Scott, and I. G. Wright, *Chem. Commun.*, 537 (1965); (c) E. S. Hall, F. McCapra, T. Money, K. Fukumoto, J. R. Hanson, B. S. Mootoo, G. T. Phillips, and A. I. Scott, *ibid.*, 348 (1966).

(2) M. Hesse, "Indolakaloide in Tabellen," Springer-Verlag, Berlin, 1964.

(3) R. H. F. Manske, Ed., "The Alkaloids," Vol. VIII, Academic Press Inc., New York, N. Y., 1965.

(4) E. Schlittler and W. I. Taylor, *Experientia*, **16**, 244 (1960).