[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

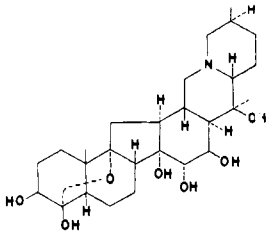
Veratrum Alkaloids. XXX.[1] The Structure and Configuration of Zygadenine[2]

BY S. MORRIS KUPCHAN

RECEIVED OCTOBER 2, 1958

The alkaloid zygadenine has been shown to have structure and configuration I. Alkaline isomerization of I leads to isozygadenine (II) and thence to pseudozygadenine (III). Acetylation of I with acetic anhydride alone leads to a triacetate (VI) and acetylation in the presence of pyridine affords a tetraacetate (VII). Acetylation of zygacine acetonide (X) gives zygadenine acetonide diacetate (XI). Mineral acid hydrolysis of XI leads to zygadenine diacetate (XII) which affords a cyclopentenone derivative (XIII) on periodate oxidation. Proof that zygadenine is 7-desoxygermine was obtained by Raney nickel desulfurization of 7-dehydrogermine 3,16-diacetate propylene thioketal hydrochloride (XV) to zygadenine 3,16-diacetate (XII).

Zygadenine is the alkamine present in three ester alkaloids which occur in zygadenus[3-7] and veratrum[8,9] species. The structure of zygadenine is of special interest in view of the powerful pharmacodynamic properties of its ester alkaloids.[10] In this paper, evidence is presented which favors assignment of structure and configuration I to zygadenine.

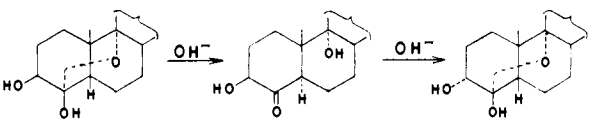


I

Zygadenine was first isolated in 1913 in a search for the poisonous principles of alkaloidal extracts of *Zygadenus intermedius* indigenous to Wyoming. A $C_{39}H_{63}O_{10}N$ formula was originally assigned to the base.[3] Subsequent re-examination of the alkamine and a series of its derivatives revealed that zygadenine has a $C_{27}H_{43}O_7N$ formula.[4]

The apparent minimal pharmacological activity of zygadenine[3] left unsolved the problem of the nature of the toxic principles of various species of zygadenus. Subsequent investigation in this Laboratory of the active principles of *Zygadenus venenosus* and *Zygadenus paniculatus* from Washington[11] showed that the toxic compounds included the zygadenine monoesters veratroylzygadenine, vanilloylzygadenine and zygacine[5-7] as well as several germine polyesters.[6,12] Veratroylzygadenine has subsequently been isolated from veratrum species known to be rich in germine polyesters.[8,9] These facts confirmed the close relationship between the two genera which was first suggested over fifty years ago on the basis of the similarity of their pharmacological actions.[13]

The isomerization triad now known to characterize the polyoxygenated veratrum alkamines (*e.g.*, veracevine[14,15] and germine[14,16]) was first recognized in the zygadenine case.[5] Furthermore, similarities in infrared spectra between the zygadenine isomers and the veracevine isomers[15] suggested quite early that there might be a close resemblance in structure among those portions of the respective molecules which are involved in the isomerization reactions. These similarities and the occurrence of zygadenine alongside germine in at least two plant genera led early to the tentative hypothesis that zygadenine contains a 3-hydroxy-4,9-hemiketal system in a molecule with the germine–veracevine skeletal system. (Independent support for the location of an oxygen function at C_9 came from another sequence; see below.) The zygadenine → isozygadenine → pseudozygadenine isomerizations would then be represented as I→II→III (*cf.* ref. 17, 18). The order of stability of the zygadenine isomers parallels that of the



I II III

veracevine series, *i.e.*, I (3-β-hydroxy-4,9-hemiketal) <II (3-β-hydroxy-4-keto-9-α-hydroxy-A/B *trans*) <III (3-α-hydroxy-4,9-hemiketal) and differs from that of the germine series.[18] This fact is compatible

(1) Part XXIX, S. M. Kupchan, THIS JOURNAL, **81**, 1921 (1959).

(2) The investigation which forms the subject of this paper was first outlined in part in a preliminary communication: *ibid.*, **78**, 3546 (1956). This work was supported by a grant (H-2275) from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

(3) F. W Heyl, F. E. Hepner and S. K. Loy, THIS JOURNAL, **35**, 258 (1913).

(4) F. W. Heyl and M. E. Herr, *ibid.*, **71**, 1751 (1949).

(5) S. M. Kupchan and C. V. Deliwala, *ibid.*, **74**, 2382 (1952); **75**, 1025 (1953).

(6) S. M. Kupchan, D. Lavie and R. D. Zonis, *ibid.*, **77**, 689 (1955).

(7) S. M. Kupchan, C. V. Deliwala and R. D. Zonis, *ibid.*, **77**, 755 (1955).

(8) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **36**, 1570 (1953).

(9) M. W. Klohs, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, THIS JOURNAL, **75**, 4925 (1953).

(10) O. Krayer, S. M. Kupchan, C. V. Deliwala and B. H. Rogers, *Arch. Exper. Path. u. Pharmakol.*, **219**, 371 (1953).

(11) *Zygadenus intermedius* (*Zygadenus venenosus gramineus*) is a species which is very closely related to *Zygadenus venenosus* which occurs in the state of Washington, according to S. J. Preece, Jr., "A Cytotaxonomic Study of the Genus Zigadenus (LILIACEAE)," Ph.D. Thesis, State College of Washington, 1956. (Available from University Microfilms, Ann Arbor, Mich., Publication no. 17,517.) I thank Mr. J. Zimmermann of the University of Wisconsin Department of Botany for calling attention to this reference.

(12) S. M. Kupchan and C. V. Deliwala, THIS JOURNAL, **74**, 3202 (1952); **76**, 5545 (1954).

(13) R. Hunt, *Am. J. Physiol.*, **6**, XIX (1902).

(14) S. W. Pelletier and W. A. Jacobs, THIS JOURNAL, **75**, 3248 (1953).

(15) S. M. Kupchan, D. Lavie, C. V. Deliwala and B. Y. A. Andoh, *ibid.*, **76**, 5519 (1953).

(16) S. M. Kupchan, M. Fieser, C. R. Narayanan, L. F. Fieser and J. Fried, *ibid.*, **77**, 5896 (1955).

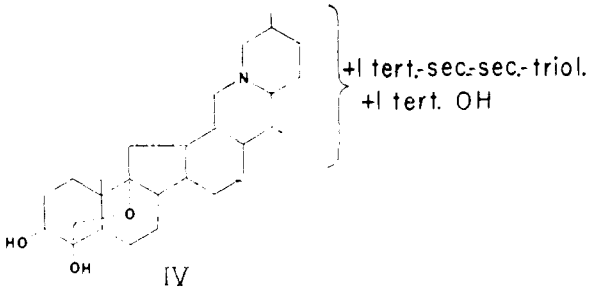
(17) D. H. R. Barton, O. Jeger, V. Prelog and R. B. Woodward, *Experientia*, **10**, 81 (1954).

(18) S. M. Kupchan and C. R. Narayanan, THIS JOURNAL, **81**, 1913 (1959).

with the view that the different order in the germine series is attributable to the effect of germine's 7-α-hydroxyl group.

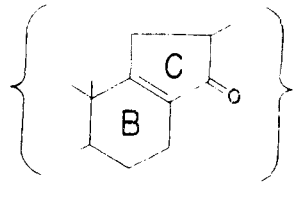
Zygadenine readily formed a triacetate upon acetylation with acetic anhydride.[6] Hence, three non-tertiary hydroxyl groups are present in the molecule. Under more vigorous acetylating conditions, zygadenine formed a tetraacetate,[6] and, by analogy with the behavior of veracevine, cevine and germine[18], it may be assumed that the fourth, more difficultly acylable, hydroxyl is the hemiketal hydroxyl.

Periodic acid titrations indicated the following uptakes: zygadenine, 3 moles; zygacine (zygadenine monoacetate[6]), 2 moles; zygadenine triacetate, 0 mole; zygacine acetonide,[6] 0 mole. These facts are most readily accommodated by the view that, like germine,[18] zygadenine contains a 1,2,3-triol system. Furthermore, the following facts show that the triol system is tertiary-secondary-secondary. Acetylation of zygacine acetonide with acetic anhydride yielded zygadenine acetonide diacetate. Mineral acid hydrolysis of the latter compound afforded zygadenine diacetate, which consumed one mole of periodic acid. These observations suggest that (1) the acetonide is tertiary-secondary, for only one of zygadenine's acylable hydroxyl groups is blocked toward acetylation and (2) the triol system is tertiary-secondary-secondary, for one of the acetate groups in zygadenine diacetate clearly occupies a terminal secondary hydroxyl group of the triol system (*cf.* ref. 18). The discussion presented thus far leads to the tentative disposition of functional groups as shown in formulation IV.



IV

The location of the triol system at C_{14}, C_{15}, C_{16} is assigned on a basis paralleling the placing of this grouping in the germine molecule. When the crude product of periodic acid oxidation of zygadenine diacetate was exposed to dilute alcoholic ammonia for fifteen minutes, a product was obtained with infrared and ultraviolet spectral properties closely paralleling those of the cyclopentenone derivative V in the germine series.[18]



V

As in the germine case, the $\Delta^{8,9}$ location of the double bond in the unsaturated ketone indicates that a tertiary hydroxyl (or equivalent) is affixed at C_9. The seventh oxygen of zygadenine is tentatively assigned to C_{20} at this point in the discussion by analogy to germine and veracevine, leading to the *structure* for zygadenine represented by I (apart from configurational relationships, for the present).

Chart 1 presents a formulation of the reactions of zygadenine based on formula I as a reasonable working hypothesis. The triacetate formed by acetic anhydride acetylation is the 3,15,16-triacetate (VI). The tetraacetate formed by acetic anhydride–pyridine acetylation is the 3,4,15,16-tetraacetate. Zygadenine acetonide is represented as the 14,15-acetonide (VIII) on the basis of the periodic acid uptakes and products discussed earlier.

Zygacine consumes two moles of periodic acid; zygacine acetonide consumes none. These facts indicate that the acetate group to zygacine is located either at C_3 or C_4. A zygadenine 4-monoacetate on acetylation with acetic anhydride alone would form zygadenine 3,4,15,16-tetraacetate (VII). In fact, zygacine is known to form zygadenine 3,15,-16-triacetate (VI) on acetylation with acetic anhydride and zygacine is therefore assigned structure IX. Zygacine acetonide is then represented as zygadenine 14,15-acetonide-3 acetate (X). The diacetate formed by acetylation of X is formulated as XI and the product of dilute mineral acid hydrolysis of XI is zygadenine 3,16-diacetate (XII). The cyclopentenone formed by periodic acid oxidation of XII followed by exposure of the initial product to dilute base now is assigned the full structure XIII. The formation of this product is readily explicable in terms of initial glycol change at C_{14}, C_{15} to a β-hydroxycyclopentanone, followed by β-elimination to XIII.

The foregoing arguments provided strong circumstantial evidence for structure I for zygadenine, but it was considered desirable to seek conclusive evidence. Proof that zygadenine is 7-desoxygermine and therefore possesses structure and configuration I was obtained by interrelation of zygadenine with germine. Treatment of 7-dehydrogermine-3,16-diacetate (XIV)[18] in methanol with 1,3-propane-dithiol and anhydrous hydrogen chloride yielded 7-dehydrogermine 3,16-diacetate-propylene thioketal hydrochloride. Raney nickel desulfurization of XV afforded zygadenine 3,16-diacetate (XII), characterized by mixed melting point and infrared spectral comparison with the authentic sample.

Experimental[19]

Periodic Acid Titrations.—The periodic acid titrations were performed as in part XXVIII.[18] Zygadenine tetraacetate, zygadenine triacetate and zygacine acetonide consumed no periodic acid in 20 hours. Zygadenine consumed 2.9 mole periodic acid in 20 hours. Zygadenine consumed 2.9 mole equivalents in 1 hour and was stable thereafter. Veratrine consumed 1.7 mole equivalents in 1 hour and was stable

(19) Melting points are corrected for stem exposure. Values of $[\alpha]_D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined in 95% ethanol on a Cary recording spectrophotometer (model 11 MS). Infrared spectra were determined in chloroform on a Baird double beam infrared recording spectrophotometer (model B). Microanalyses were carried out by Dr. S. M. Nagy and his associates at M.I.T. on samples dried *in vacuo* at 110°.

CHART 1

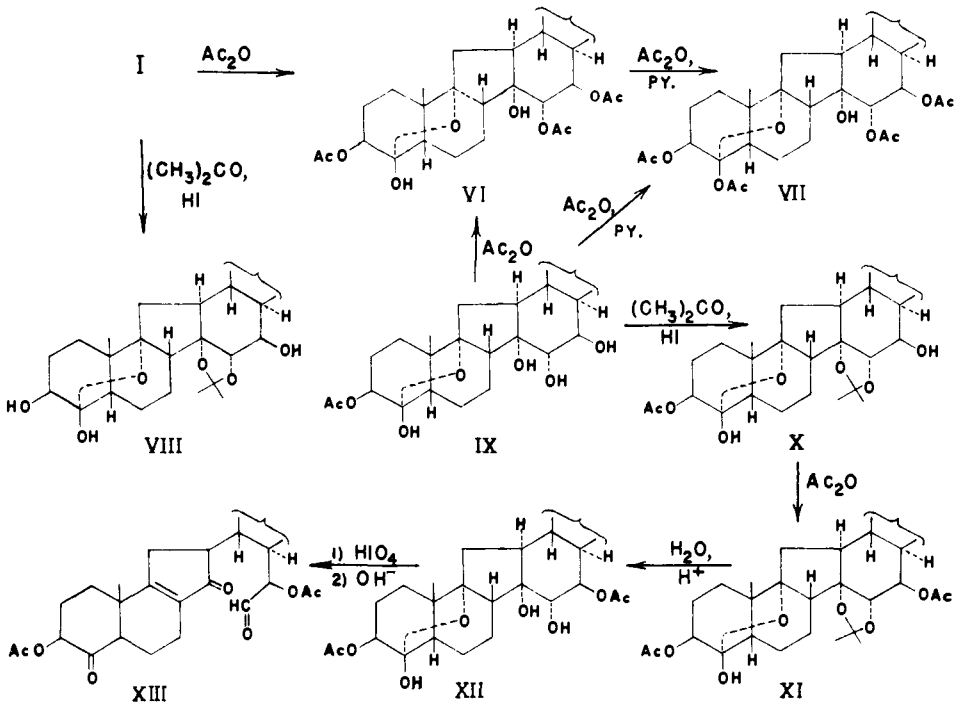
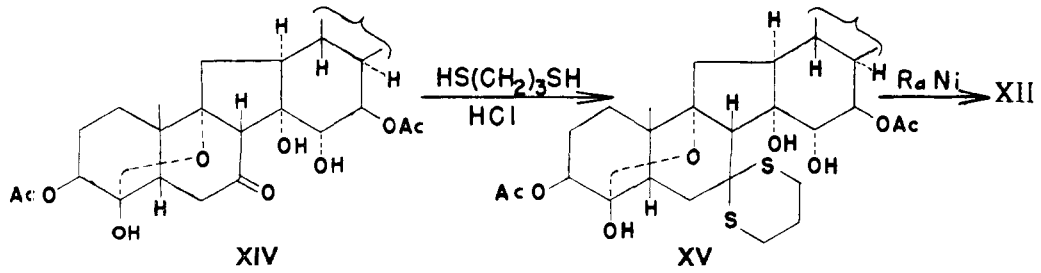


CHART 2



thereafter. Zygadenine diacetate took 1.1 mole equivalents in 1 hour and was stable thereafter.

Zygadenine Acetonide Diacetate (XI).—Zygacine acetonide[6] (1.25 g.) was dissolved in acetic anhydride (15 ml.) and the solution was heated on the steam-bath for 2 hours. The excess acetic anhydride was decomposed by cautious addition of methanol (20 ml.) and the solution was evaporated to dryness *in vacuo*. The residue was treated with water (5 ml.) and ammonium hydroxide (to *p*H 8.5) and the solution was extracted with chloroform (nine 10-ml. portions). The combined chloroform extract was washed with water (10 ml.), dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue crystallized from ether; yield 1.07 g., m.p. 269–271° dec. Recrystallization from acetone gave clusters of needles (810 mg.), m.p. 271–272° dec., $[\alpha]^{23}D$ −29° (*c* 1.19, pyr.).

Anal. Calcd. for $C_{30}H_{45}O_7N(COCH_3)_2$: C, 66.10; H, 8.32; acetyl, 13.93. Found: C, 66.33; H, 8.35; acetyl, 13.61.

Zygadenine Diacetate (XII).—Zygadenine acetonide diacetate (500 mg.) was dissolved in acetic acid (4 ml.) and 1:4 dilute hydrochloric acid (10 ml.) and the solution was allowed to stand at room temperature for 4 hours. Dilute ammonium hydroxide was added to *p*H 8 and the solution was extracted with chloroform (ten 15-ml. portions). The chloroform was dried over sodium sulfate and brought to dryness *in vacuo*. The residue crystallized readily from acetone–ether; yield 220 mg., m.p. 252–255° dec. Recrystallization from acetone–ether gave clusters of rods (110 mg.), m.p. 255–257° dec., $[\alpha]^{23}D$ −50° (*c* 1.14, pyr.).

Anal. Calcd. for $C_{27}H_{41}O_7N(COCH_3)_2$: C, 64.45; H, 8.20; acetyl, 14.90; equiv. wt., 578. Found: C, 64.40; H, 8.20; acetyl, 14.83; equiv. wt., 582.

Periodic Acid Oxidation of Zygadenine Diacetate.—Zygadenine diacetate (30 mg.) was dissolved in 5% acetic acid (2 ml.) and 0.02 *M* periodic acid (5 ml.) and water (3 ml.) were added. After one hour, a 2-ml. aliquot was taken and titrated in the usual manner; periodic acid consumption was found to be 1.0 mole equivalent. The remaining solution was made alkaline with dilute ammonium hydroxide and extracted with chloroform (eight 10-ml. portions). Evaporation of the chloroform solution to dryness left an amorphous residue (10 mg.). This material showed no ultraviolet absorption other than the high end absorption characteristic of these alkaloids. The solid was dissolved in alcohol (2 ml.) and ammonium hydroxide (0.04 ml.) was added. The solution was allowed to stand for 3 hours at room temperature. The solution now showed intense absorption at 238 mμ. Evaporation of the solution to dryness in a stream of air left a residue which showed infrared absorption peaks at 2.90, 5.78–5.85, 5.92 and 6.05 μ. The infrared and ultraviolet spectral properties parallel closely those of the cyclopentenone derivative derived from germine diacetate.[18]

7-Dehydrogermine 3,16-Diacetate Propylene Thioketal Hydrochloride (XV).—To a saturated solution of hydrogen chloride in methanol (15 ml.) at −15° was added 7-dehydrogermine diacetate[18] (2.0 g.) and 1,3-propanedithiol (7 ml.). Hydrogen chloride gas was bubbled through the solution for 1 hour and the solution was then allowed to stand for 2 additional hours at −15°. Chloroform (100 ml.) was added, and then solid sodium bicarbonate was added portionwise to cessation of bubbling. The precipitated salts were filtered and the yellow filtrate evaporated to dryness *in vacuo*. Solution of the residue in chloroform led to separation of unidentified solid material (850 mg.). Evaporation of the chloroform filtrate to dryness and crystallization of the resi-

due from acetone yielded a second solid product (900 mg.). Two recrystallizations of the second product from alcohol–ether gave colorless rods (740 mg.), m.p. 265–266° dec., $[\alpha]^{23}D$ −5° (*c* 1.25, pyr.), λ_{max} 246 mμ (ε 600).[20]

Anal. Calcd. for $C_{34}H_{51}O_9NS_2 \cdot HCl$: C, 56.84; H, 7.30; S, 8.93; Cl, 4.94. Found: C, 56.86; H, 7.80; S, 9.13; Cl, 5.11.

Desulfurization of 7-Dehydrogermine 3,16-Diacetate Propylene Thioketal Hydrochloride.—To a solution of 7-dehydrogermine 3,16-diacetate propylene thioketal hydrochloride (275 mg.) in alcohol (30 ml.) was added Raney nickel (3 g.) and the solution was heated under reflux for 11 hours. The suspension was filtered and the Raney nickel extracted twice with hot alcohol. The alcoholic solutions were combined and evaporated to dryness *in vacuo*. The residue crystallized slowly from acetone–ether; yield 30 mg. of rods, m.p. 253–255°. The melting point was not depressed by admixture of an authentic sample of zygadenine 3,16-diacetate and the infrared spectra of the respective samples were identical.

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(20) *Cf.* D. J. Cram and M. Cordon, THIS JOURNAL, **77**, 1810 (1955).

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT OF CIBA PHARMACEUTICAL PRODUCTS, INC.]

Rauwolfia Alkaloids. XXXI.[1] The Synthesis and Activity of Some Reserpine Analogs[2]

BY R. A. LUCAS, M. E. KUEHNE, M. J. CEGLOWSKI, R. L. DZIEMIAN AND H. B. MACPHILLAMY

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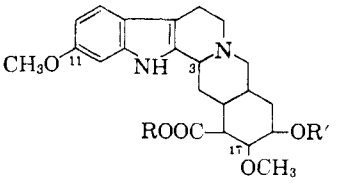
In an effort to separate the medically important hypotensive and sedative components of the action characteristic of reserpine, over 100 derivatives of methyl reserpate were prepared. Of these, two were outstanding; the carbethoxysyringate ester having a predominantly hypotensive effect with little sedation, and the 3-dimethylaminobenzoate ester which proved to be a fast acting sedative with little effect on the blood pressure. The physical properties and methods of preparation of the various derivatives are described.

The medical importance of the Rauwolfia alkaloid reserpine has stimulated considerable interest in determining the effect that variations in the chemical structure of this molecule might have upon the biological activity of the drug. It would be of theoretical interest if an analog of reserpine could be prepared with an activity such that either the hypotensive or sedative component of the action characteristic of reserpine predominated. This would then demonstrate that these actions, which have not previously been separated, could be dissociated. Such substances could also be of practical importance for in some clinical cases of hypertension the concomitant sedation produced by reserpine may not be desirable. While this side reaction can usually be adequately controlled by regulation of the dosage, a reserpine derivative with a hypotensive action considerably greater than its sedative effect would be clinically useful. On the other hand, the value of reserpine as a tranquilizer would be markedly increased if an analog could be prepared which had a strong sedative action of rapid onset. A de-emphasis of the hypotensive action in such a product would be desirable but not essential since reserpine does not lower the blood pressure of normotensive patients. With these considerations in mind we set out to prepare some substances derived from reserpine which might fulfill the above conditions.

Some information was already available on the biological effect of structural changes in the reserpine molecule. The naturally occurring 11-desmethoxy compound deserpidine[3] and the 11,17-didesmethoxy analog[4] both had typical reserpine-like activity. Substitution of the indole nitrogen with methyl and allyl groups[5] produced substances totally lacking in reserpine-like action. In fact their mild stimulant effect could be considered as antagonistic to reserpine. The N-oxide of the other more basic nitrogen in reserpine has a reserpine-like activity,[6] but the quaternary salt[7] was found to be completely inactive. This was also true of such degradation products as methyl reserpate, reserpic acid and its lactone.

Since none of the above-mentioned compounds showed any indication of an action which was either predominantly hypotensive or sedative, we initiated a program for the synthesis of reserpine analogs in which the R and R′ groups were varied.



Reserpine,

R = CH_3

R′ = $-CO-C_6H_2(OCH_3)_3$

We had previously prepared the compound in which R = ethyl and R′ = trimethoxybenzoyl[7] and this was found to have an activity comparable to reserpine. However, further variants in this direction were limited by the relative difficulty of preparation of the corresponding reserpic acid esters. The use of simple acid-catalyzed esterification is complicated by the known facile epimerization of reserpine derivatives at C-3 to the corresponding inactive iso-compounds[8] under acid conditions and is, therefore, not too adaptable. The action of reserpic acid lactone with alcohols did not give satisfactory yields of esters. The only sure method was that using the diazoalkanes, a

(1) Part XXX, J. M. Müller, *Experientia*, **13**, 479 (1957).

(2) Presented in part before the Division of Medicinal Chemistry at the 134th National Meeting of the American Chemical Society, Chicago, Ill., September 8, 1958.

(3) For a complete review of the Rauwolfia alkaloids see R. E. Woodson, Jr., H. W. Youngken, E. Schlittler and J. A. Schneider "Rauwolfia," Little, Brown and Co., Boston, Mass., 1957.

(4) F. L. Weisenborn, THIS JOURNAL, **79**, 4818 (1957).

(5) C. F. Huebner, *ibid.*, **76**, 5792 (1954).

(6) P. R. Ulshafer, W. I. Taylor and R. H. Nugent, *Compt. rend.*, **244**, 2988 (1957).

(7) L. Dorfman, *et al.*, *Helv. Chim. Acta*, **37**, 59 (1954).

(8) H. B. MacPhillamy, *et al.*, THIS JOURNAL, **77**, 1071, 4335 (1955).