TABLE I

PROPERTIES OF 1,3,5-TRINITROBENZENE ADDUCTS OF INDOLE COMPOUNDS

Trinitrobenzene derivative of	M.p., °C.	Color	Formula	Dumas Nitrogen Calc'd	Dumas Nitrogen Found
4-Chloro-3-indoleacetic acid	209	Orange	$C_{16}H_{11}ClN_4O_8$	13.3	13.4
5-Chloro-3-indoleacetic acid	202	Yellow	$C_{16}H_{11}ClN_4O_8$	13.3	13.2
6-Chloro-3-indoleacetic acid	160	Yellow	$C_{16}H_{11}ClN_4O_8$	13.3	13.4
7-Chloro-3-indoleacetic acid	182	Yellow	$C_{16}H_{11}ClN_4O_8$	13.3	13.2
5,7-Dichloro-3-indoleacetic acid	155	Yellow	$C_{16}H_{10}Cl_2N_4O_8$	12.3	12.5
4,7-Dichloro-2-methyl-3-indoleacetic acid	232	Yellow	$C_{17}H_{12}Cl_2N_4O_8$	11.9	11.8
3-Indoleacetic acid hydrazide	169	Yellow	$C_{16}H_{15}N_6O_7$	20.8	20.8
3-Indoleacetamide	165	Orange	$C_{16}H_{13}N_5O_7$	18.1	18.1
3-Indoleacetonitrile	136	Yellow	$C_{16}H_{11}N_5O_6$	19.0	19.0
3-Indoleacetic hydroxamic acid	144	Brown	$C_{16}H_{13}N_5O_8$	17.4	17.2
3-Indolealdehyde thiosemicarbazone	196	Red	$C_{16}H_{13}N_7O_6S$	22.6	22.6
n-Hexyl-3-indoleacetate	91	Yellow	$C_{22}H_{24}N_4O_8$	11.9	11.8
n-Heptyl-3-indoleacetate	93	Yellow	$C_{23}H_{26}N_4O_8$	11.5	11.7
n-Octyl-3-indoleacetate	87	Yellow	$C_{24}H_{28}N_4O_8$	11.2	11.3
n-Nonyl-3-indoleacetate	97	Yellow	$C_{25}H_{30}N_4O_8$	10.9	11.1
n-Decyl-3-indoleacetate	92	Yellow	$C_{26}H_{32}N_4O_8$	10.6	10.5
n-Undecyl-3-indoleacetate	102	Yellow	$C_{27}H_{34}N_4O_8$	10.3	10.5
n-Dodecyl-3-indoleacetate	97	Yellow	$C_{28}H_{36}N_4O_8$	10.1	10.2
n-Tetradecyl-3-indoleacetate	96	Yellow	$C_{30}H_{40}N_4O_8$	9.6	9.5
n-Hexadecyl-3-indoleacetate	96	Yellow	$C_{32}H_{44}N_4O_8$	9.1	9.4
Carbazole	195	Orange	$C_{18}H_{12}N_4O_6$	14.7	14.7
1,3-Dimethylindole	169	Orange	$C_{16}H_{14}N_4O_6$	15.6	15.6
Trimethyl skatyl ammonium methyl sulfate	133	Yellow	$C_{19}H_{23}N_5O_{10}$	13.6	13.4
Gramine	117	Orange	$C_{17}H_{17}N_5O_6$	18.1	17.8
3-Indoleacetyl chloride	88	Yellow	$C_{16}H_{11}ClN_4O_7$	13.8	13.7

The properties of 25 new derivatives, prepared as previously described,[2,4] are summarized in Table I.

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(4) J. J. Sudborough, *J. Chem. Soc.*, **109**, 1339 (1916).

The Nature of Serpine

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Received July 20, 1956

Serpine is one of the twenty or more alkaloids isolated from the Indian Apocyanaceae, *Rauwolfia serpentina* Benth. This alkaloid, described by Chatterjee and Bose,[1] was believed to be a stereoisomer of yohimbine on the basis of preliminary studies on the small amount of material available. The isolation of larger quantities of this substance from the Central American species *Rauwolfia heterophylla*, a fortunate choice of paper chromatographic systems,[2] and the usefulness of infrared spectra in the identification of small quantities of material has enabled us to ascertain that serpine is not a pure substance, but a mixture of two stereoisomeric alkaloids, rauwolscine and yohimbine.

Neither serpine base nor the oxalate salt were found to be separable into its components by crystallization from a variety of solvents nor by chromatography on alumina. The new alkaloid mixture shows a constant sharp melting point of about 213–215° (dec.) over a rather broad range of composition, when the proportions are estimated by optical rotation. However, rauwolscine picrate is much less soluble in aqueous methanol than is yohimbine picrate. Utilization of this property has enabled us to separate serpine into pure yohimbine and pure rauwolscine. We wish therefore to suggest that the name serpine be dropped from the literature.

Added in press. Dr. Norbert Neuss informs us that his studies at the Lilly Research Laboratories indicate that serpine is not a simple mixture of yohimbine and rauwolscine, but a "mixed crystal". Thus the powder x-ray diagram of this alkaloid does not show lines of either of the two components. A summation infrared spectrum of equimolar proportions of yohimbine and rauwolscine in chloroform solution was identical in every respect with that of serpine.

EXPERIMENTAL

All infrared spectra were determined on potassium bromide pellets. Melting points are corrected.

Serpine from Rauwolfia heterophylla. A solution of 40 g. of crude *R. heterophylla* alkaloids[3] from 15 Kg. of root was dissolved in 300 ml. of chloroform, and extracted with four

(1) A. Chatterjee and S. Bose, *Experientia*, **10**, 246 (1954).
(2) F. A. Hochstein, K. Murai, and W. H. Boegemann, *J. Am. Chem. Soc.*, **77**, 3551 (1955).

(3) The plant material used for this study was obtained from a commercial source. We are indebted to Dr. L. Nickell for confirming the botanical identity of the material.

100-ml. portions of 5% acetic acid. The acetic acid extract was adjusted to *p*H 7.5, and 8 g. of precipitated amorphous alkaloids were separated by filtration. This product was dissolved in 60 ml. of ethanol, and 4 g. of oxalic acid in 20 ml. of hot ethanol was added. After standing overnight, a colorless crop of fine needles precipitated. These crude crystals were digested in 130 ml. of hot ethanol for 20 minutes to yield larger crystals, Wt. 4.0 g., m.p. 265–266° (dec.). Recrystallization from hot ethanol did not change the melting point. An 0.8-g. portion of this material was converted to the free base and was crystallized twice from benzene to yield 0.48 g. of colorless crystals, m.p. 213–214° (d.) $[\alpha]_D^{25}$ +5.6° (c, 1, $CHCl_3$). The infrared spectrum of this material was identical to that of serpine from *R. serpentina* (m.p. 213°) $[\alpha]_D^{25}$ +70° (pyridine),[1] and like serpine, it showed four spots on chromatography on formamide-impregnated paper, with benzene-chloroform as the developing solvent. Two major spots, R_f = 0.40 and 0.62, were identical with yohimbine and rauwolscine respectively, while two minor spots, of slower R_f appeared to be due to traces of other alkaloids. Four recrystallizations of this product from benzene raised the melting point somewhat to 217–219° (dec.), without significantly changing the paper chromatographic picture.

Separation of serpine from R. heterophylla into yohimbine and rauwolscine. A 0.2-g. portion of serpine, from *R. heterophylla*, m.p. 217–219°, was dissolved in 0.5 ml. of hot methanol, and mixed with 0.17 g. of picric acid in 2 ml. of hot methanol. Water, 0.25 ml. was added to incipient turbidity. The crystalline picrate which separated (0.15 g.) melted at 191–195° (dec.). The picrate was converted to the free base, and crystallized from benzene, and then from aqueous ethanol to yield about 100 mg. of the characteristic twinned prisms of rauwolscine m.p. 233–235° (dec.) mixture m.p. with an authentic sample of rauwolscine, m.p. 233–235°, not depressed. The infrared spectrum and R_f on paper chromatography (0.62) were also identical to those of pure rauwolscine. The hydrochloride salt, which separated from aqueous acid as long needles, had m.p. 268–270° (dec.) $[\alpha]_D^{25}$ +70° (0.5 in water).

The mother liquors from the preparation of the picrate were converted to the base, and crystallized from benzene-ligroin, then from aqueous methanol to yield about 20 mg. of yohimbine, m.p. 220–222°, mixture m.p. with an authentic sample, m.p. 221–223°, not depressed. The R_f on paper chromatography, 0.40 and the infrared spectrum were identical to those of authentic yohimbine.

Separation of serpine from R. serpentina into yohimbine and rauwolscine. A 10-mg. portion of serpine[1] (obtained from Dr. A. Chatterjee) m.p. 213–214° dissolved in 0.1 ml. of methanol was mixed with 9 mg. of picric acid in 0.2 ml. of methanol; then 0.05 ml. of water was added, and the solution was left overnight at room temperature. The crystalline precipitate was separated by filtration, suspended in 0.5 ml. of chloroform, and washed with 0.5 ml. of 5% ammonium hydroxide to convert it to the base. The chloroform solution was concentrated to dryness, and the amorphous residue was crystallized from aqueous methanol to yield about 2 mg. of colorless crystals of rauwolscine, m.p. 230–233° (Kofler hot stage) mixture m.p. with an authentic sample, m.p. 236–238° not depressed. The infrared spectrum and paper chromatographic behaviour of this material were identical to those of pure rauwolscine.

The mother liquors from the isolation of the rauwolscine picrate were converted to the base, and the amorphous base was crystallized from aqueous methanol to yield about 1 mg. of yohimbine, m.p. 210–218° (Kofler hot stage); mixture m.p. with an authentic sample, m.p. 223–224°, not depressed. The infrared absorption spectrum and behavior on paper chromatography were identical to those of authentic yohimbine, though the melting point suggests that the preparation was not pure.

Acknowledgment. We are greatly indebted to Dr. A. Chatterjee, who made this study possible by providing authentic rauwolscine and serpine. We also wish to acknowledge the interest and suggestions of Dr. C. Djerassi. We are indebted to Mrs. A. Paradies, for her very capable help with this study, and to Mr. W. Boegemann for the paper chromatographic studies.

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The Oxidation of Cholesterol by Periodic Acid

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Received July 20, 1956

The recent appearance of an article by Chatterjee and Majumdar,[1] describing the cleavage oxidation of certain isolated and terminal double bonds by periodic acid, prompts us to report findings of a related nature arising from a study of the effect of this reagent on steroidal olefins. Our observations revealed that the Δ^5 double bond of the steroid nucleus may respond more or less specifically to the oxidizing action of periodic acid and this reagent, therefore, may be of diagnostic value for this grouping.[2]

Periodic acid has generally been considered to be a specific reagent for the cleavage oxidation of glycol and ketol systems. However, we have found that cholesterol (I) is oxidized at room temperature by periodic acid in aqueous tetrahydrofuran to give cholestane-3β,5α,6β-triol (III) in 60% isolated yield. Moreover, by greatly increasing the reagent concentration and extending the time interval of reaction the concomitant formation of 3β,5α-dihydroxycholestane-6-one (V) also occurred.

A rate study run under the optimum conditions found for producing the triol indicated that one mole of periodate was consumed per mole of steroid and that the reaction was essentially complete in 48 hours. Heating accelerated the oxidation reaction but gave an inferior melting product.

The periodate oxidation of cholesterol probably proceeds by way of a 5,6-oxido derivative (II) as suggested by the Indian authors to explain their particular findings. This route further would be compatible with the known conversion in high yield of cholesterol oxide to III by periodic acid

(1) Chatterjee and Majumdar, *Anal. Chem.*, **28**, 878 (1956).

(2) For example $\Delta^{9(11)}$-dehydrotigogenin acetate and $\Delta^{9(11)}$-anhydrohydrocortisone acetate were not functionally affected by this reagent.