to +500°) than for the corresponding halogen-free steroids (+190° to +230°), while the reverse is true for the fluoro-compounds (−10° to 40°).

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ENZYMATIC FORMATION OF CORTICOSTEROID GLUCURONIDES[1]

Sir:

The metabolites of steroid[2a] and thyroid[2b,c] hormones are known to be excreted in significant amounts as conjugates of glucuronic acid. In previous studies, glucuronide synthesis of *o*-aminophenol and menthol has been observed in cell free preparations of liver and shown to require the presence of uridine diphosphate glucuronic acid (UDPGA).[3]

We should like to report the enzymatic formation of glucuronides of corticosteroids, such as tetrahydrocortisone.[4] This reaction is catalyzed by an enzyme system in the microsomes of mammalian liver of several species and also requires UDPGA as the glucuronide donor. In addition to tetrahydrocortisone glucuronide, uridine diphosphate (UDP) is a product of the conjugation (Table I). The reaction would appear to proceed as shown

UDPGA + Tetrahydrocortisone ⟶
Tetrahydrocortisone glucuronide + UDP

Attempts to demonstrate reversibility of this reaction have thus far been unsuccessful.

Incubation of tetrahydrocortisone with microsomes of guinea pig liver and UDPGA resulted in disappearance of free tetrahydrocortisone (extracted with methylene chloride and measured by the method of Porter and Silber[5]) which was not observed in the absence of UDPGA, or when UDPGA was replaced by uridine diphosphate glucose (UDPG) or glucuronolactone (Table I). After extraction with methylene chloride, the aqueous residue (adjusted to *p*H 2–3) was treated with butanol[6] to extract any steroid glucuronide present. This butanol fraction, after distillation *in vacuo*, yielded Porter–Silber reacting material and gave a positive carbazole reaction for glucuronic acid.[7] Alternatively, when the aqueous residue was incubated with purified bacterial β-glucuronidase,[8] subsequent re-extraction with methylene chloride resulted in almost quantitative recovery[5] of a steroid with an R_f corresponding to tetrahydrocortisone on paper chromatography (modified benzene–aqueous methanol system of Bush[9]). These observations were taken as evidence that the free tetrahydrocortisone, which disappeared upon incubation in the complete system, was converted to tetrahydrocortisone glucuronide.

Similar results have been obtained with tetrahydrohydrocortisone as the substrate. However, cortisone is not metabolized in this system. This would suggest that reduction of the 3-ketone to the 3-hydroxyl group is necessary for conjugation to occur and that the probable site of coupling on the steroid molecule is at the 3-hydroxyl position, as in the case of pregnanediol 3-β-*d*-glucuronide.[10]

The appearance of UDP in the reaction mixture was detected by means of paper chromatography.[11] UDP was also measured spectrophotometrically by following the disappearance of reduced diphosphopyridine nucleotide (DPNH) in the phosphopyruvate–pyruvate phosphokinase system coupled to the DPNH-lactic dehydrogenase system.[12]

TABLE I

ENZYMATIC SYNTHESIS OF TETRAHYDROCORTISONE GLUCURONIDE

Reaction mixture consisted of 0.2 ml. 0.5*M* phosphate buffer *p*H 7.4; 0.2 μM. tetrahydrocortisone; 1 ml. guinea pig liver microsomal preparation (30 mg. protein per ml.); total volume 3 ml.; incubated 30 min., at 38° in air; additions as indicated below.

Additions, μM	μM Tetrahydrocortisone Disappearing on incubation[a]	Recovered with β-glucuronidase[b]	μM UDP formed[c]
UDPGA[d] (0.065)	0.046	0.042	0.040
UDPG (0.1)	0.000	0.000	0.000
Glucuronolactone (0.1)	0.000	0.000	0.000

[a] Steroid determined in CH_3Cl extracts before and after incubation by the method of Porter and Silber.[5] [b] Reaction mixture extracted after incubation with CH_3Cl to remove free steroid; aqueous residue then incubated with β-glucuronidase (40 units per ml.) at 37° for 36 hours; steroid extracted again with CH_3Cl and measured as in footnote.[a] [c] Determined spectrophotometrically with pyruvate phosphokinase and lactic dehydrogenase.[12] [d] Generated enzymatically[3d] from UDPG with UDPG dehydrogenase and DPN^+.

In addition to the formation of corticosteroid glucuronides, we have obtained preliminary evidence for the synthesis of glucuronides of phenolphthalein and thyroxine by the enzyme system described. Disappearance of phenolphthalein occurred upon incubation and was detected by the reduction in the optical density at 540 mμ (in alkali). Subsequent hydrolysis with β-glucuronidase resulted in a return of the optical density to its original value. When I^{131}-labeled L-thyroxine was

(1) We wish to express our appreciation to Dr. Jack L. Strominger and Dr. Herman M. Kalckar for the mauy helpful suggestions throughout this study, and for their generous supply of UDPG and UDPG dehydrogenase; and to Dr. Gordon Tomkins for his constant and valuable advice.

(2) (a) For references see L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publ. Corp., New York, N. Y., 1949; (b) A. Taurog, F. N. Briggs, and I. L. Chaikoff, *J. Biol. Chem.*, **194**, 655 (1952); (c) J. Roche, O. Michel, R. Michel, and J. Tata, *Biochem. Biophys. Acta*, **13**, 471 (1954).

(3) (a) G. J. Dutton and I. D. E. Storey, *Biochem. J.*, **53**, xxxvii (1953); (b) **57**, 275 (1954); (c) E. E. B. Smith and G. T. Mills, *Biochem. Biophys. Acta*, **13**, 386 (1954); (d) J. L. Strominger, H. M. Kalckar, J. Axelrod and E. S. Maxwell, THIS JOURNAL, **76**, 6411 (1954).

(4) Tetrahydrocortisone = pregnane-3α,17α,21-triol-11,20-dione; tetrahydro-hydrocortisone = pregnane-3α,11β,17α,21-tetrol-20-one; cortisone = Δ⁴-pregnene-17,21-diol-3,11,20-trione.

(5) C. C. Porter and R. H. Silber, *J. Biol. Chem.*, **185**, 201 (1950); R. H. Silber and C. C. Porter, *ibid.*, **210**, 923 (1954).

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the substrate and the reaction mixture chromatographed (collidine–NH_3–water), a metabolite (R_f 0.15) migrating slower than thyroxine (R_f 0.49) was detected by radioautography. Thyroxine was liberated from this compound after treatment with β-glucuronidase. In its chromatographic behavior and the results of enzymatic hydrolysis, this metabolite resembles the thyroxine conjugate found in rat bile by Taurog, *et al.*[2b] (Cpd. U), and Roche, *et al.*[2c] (Cpd. A), and considered by them to be thyroxine glucuronide.

The glucuronide conjugating reaction involving UDPGA and the microsomal system appears to be a general mechanism for the formation of phenolic and alcoholic glucuronides. However, demonstration of the specificity of the enzyme system involved must await purification of the microsomal preparation.

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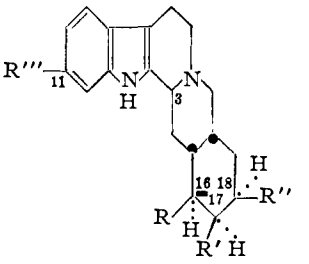
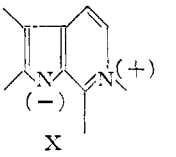
RAUWOLFIA ALKALOIDS. XVIII.[1] ON THE CONSTITUTION OF DESERPIDINE AND RESERPINE

Sir:

In a recent publication[2] we have proposed a structure for deserpidine, a minor alkaloid of many Rauwolfia species, and have pointed out its close chemical and biological similarly to reserpine.[3] We have now been able to convert this alkaloid to α-yohimbine (rauwolscine)[4] by the following series of reactions. Methyl deserpidate tosylate (II) on treatment with sodium iodide or lithium bromide yielded the corresponding 18-halogen compounds III (bromide, m.p. 179–182°, calcd. for $C_{22}H_{27}BrN_2O_3$: C, 59.06; H, 6.08; N, 6.26. Found: C, 58.98; H, 6.31; N, 6.43). These we dehalogenated with zinc in acetic acid to the 18-desoxy compound IV, m.p. 272–275°, (calcd. for $C_{22}H_{28}N_2O_3$: C, 71.71; H, 7.66; N, 7.60. Found: C, 71.94; H, 7.49; N, 7.67). Cleavage of the 17-methoxy group with hydrobromic acid and reesterification of the resulting hydroxyamino acid V with diazomethane afforded α-yohimbine (VI), m.p. 240–242° $[\alpha]^{23}D$ −22 ± 2 (ethanol) (calcd. for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.08; H, 6.96; N, 7.77). The infrared spectrum was identical with that of α-yohimbine isolated from *Rauwolfia canescens* leaves.[5]

A further linking of the two alkaloids was accomplished by treatment of the tosylate II with lithium aluminum hydride to form deserpidinol VII, m.p. 232–236° (calcd. for $C_{21}H_{28}N_2O_2$: C, 74.08; H, 8.29; N, 8.23. Found: C, 73.78; H, 8.38; N, 8.29). This compound when subjected to ether cleavage gave rauwolscinyl alcohol (VIII),[6] m.p. 229–231° (calcd. for $C_{20}H_{26}N_2O_2$: C, 73.59; H, 8.03. Found: C, 73.55; H, 7.96); infrared spectrum identical with that of a sample of rauwolscinyl alcohol prepared by the reduction of α-yohimbine (rauwolscine).





X

	R	R′	R″	R‴	C-3
I	$COOCH_3$	OCH_3	3,4,5-Trimethoxybenzoyloxy	H	α
II	$COOCH_3$	OCH_3	Tosyloxy	H	α
III	$COOCH_3$	OCH_3	Br or I	H	α
IV	$COOCH_3$	OCH_3	H	H	α
V	COOH	OH	H	H	β
VI	$COOCH_3$	OH	H	H	β
VII	CH_2OH	OCH_3	H	H	α
VIII	CH_2OH	OH	H	H	β
IX	CH_2OH	OCH_3	OH	OCH_3	α
XI	CH_2OH	OCH_3	OH	OCH_3	β

Since α-yohimbine has been shown to have the alloyohimbane structure[7] it would seem logical on the basis of the above interconversion to assign the same stereochemical configuration to the basic ring system of deserpidine. However we have accumulated considerable evidence which shows that resperine and its derivatives, (methyl reserpate, reserpinediol, reserpinol) and with less ease also deserpidine and its derivatives undergo an acid or base catalyzed epimerization at the C_3 center. That this and only this center is involved is shown by the successive lead tetraacetate oxidation of (for example) reserpinediol (IX) to tetradehydroreserpinediol (X) [isolated as the hydrochloride: m.p. 280–282°; calcd. for $C_{22}H_{27}N_2O_4Cl\cdot{}^1/_2H_2O$: C, 61.75; H, 6.34; N, 6.55. Found: C, 62.07; H, 6.63; N, 6.59; ultraviolet maxima at 251–2 mμ (log ε 4.52) and 325 mμ (log ε 4.33)] and reduction with sodium borohydride to 3-iso-reserpinediol (XI), m.p. 220–222° (calcd. for $C_{22}H_{30}N_2O_4\cdot{}^1/_2H_2O$: C, 66.83; H, 7.59. Found: C, 66.35; H, 8.06. Diacetate: m.p. 210–212°, calcd. for $C_{26}H_{34}N_2O_6$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.40; H, 7.41; N, 5.91), which was identical with a sample prepared by the acid or base catalyzed epimerization of reserpinediol. Therefore, it seemed probable that in the conversion of deserpidine to α-yohimbine inversion has taken place. Evidence to show that this was actually so was obtained by treating 3-epi-α-yohimbine[1] under the demethylation condition described above. After reesterification we obtained α-yohimbine, a transformation which has also been accomplished by oxidation and subsequent reduction of ring C[1].

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