

compound was not detectable in the plant extracts using TLC.

This communication is the first report of the occurrence of this simple β -phenethylamine in nature. Considering its relatively high concentration and structural similarity to other physiologically active alkaloids, normacromerine may be at least partially responsible for the psychotropic effects attributed to the plant.

(1) J. F. Hodgkins, S. D. Brown, and J. L. Massingill, *Tetrahedron Lett.*, **14**, 1321(1967).

(2) L. E. Below, A. Y. Leung, J. L. McLaughlin, and A. G. Paul, *J. Pharm. Sci.*, **57**, 515(1968).

(3) L. Benson, *Cact. Succ. J.*, **41**, 185(1969).

(4) S. Agurell, *Lloydia*, **32**, 206(1969).

(5) S. Agurell, *Experientia*, **25**, 1132(1969).

(6) M. J. Superweed, "Herbal Highs," Stone Kingdom Syndicate, San Francisco, Calif., 1970, p. 5.

(7) J. L. McLaughlin and A. G. Paul, *Lloydia*, **29**, 315(1966).

(8) D. L. Braga and J. L. McLaughlin, *Planta Med.*, **17**, 87(1969).

(9) W. W. Speir, V. Mhramian, and J. L. McLaughlin, *Lloydia*, **33**, 15(1970).

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Received July 22, 1971.

Accepted for publication August 30, 1971.

Supported by U. S. Public Health Service Grant MH-17128-03 from the National Institute of Mental Health.

W. J. Keller acknowledges the Sydnor Barksdale Penick Memorial Fellowship from the American Foundation for Pharmaceutical Education and an assistantship from the University of Washington Graduate School Research Fund.

The authors thank Dr. E. F. Anderson for confirming the plant identification and Dr. A. T. Shulgin for samples of reference compounds.

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Facile Differential UV Determination of Steroids with Conjugated Ketone Chromophores *via* Lithium Borohydride Reduction

Keyphrases \square Steroids, conjugated keto—identification *via* lithium borohydride reduction in tetrahydrofuran, differential UV determination \square Lithium borohydride reduction in tetrahydrofuran—determination of conjugated ketosteroids, differential UV spectrophotometry \square UV spectrophotometry, differential—determination of conjugated ketosteroids *via* lithium borohydride reduction

Sir:

Many corticosteroids, progestins, and androgenic steroid drugs have 3-keto functions conjugated with double bonds. The ketone moiety in these conjugated

Table I—Reduction of Conjugated 3-Ketosteroids with LiBH_4 in Tetrahydrofuran

Steroid	Type ^a	Direct UV (in Methanol)		Differential (Procedure)	
		$\lambda_{\text{max.}}$, nm.	<i>a</i>	$\lambda_{\text{max.}}$, nm.	<i>a</i>
Cortisone	I	241	41.5	241	40.4
Cortisone acetate	I	241	38.6	241	38.0
Hydrocortisone	I	241	42.6	241	41.9
Progesterone	I	241	54.0	241	51.0
Chlormadinone acetate	II	284	49.7	284	49.7
Betamethasone	III	239	40.4	239	37.2
Triamcinolone	III	240	37.0	240	35.9

^a Type I = 4-en-3-one, II = 4,6-dien-3-one, and III = 1,4-dien-3-one.

systems is reduced to hydroxyl by metal borohydrides, thereby abolishing the conjugation and the strong UV absorption which result from it. Several workers (1-5) exploited this reaction in the selective analysis of steroids. Görög (5) described an elegant differential UV spectrophotometric method for conjugated 3-ketosteroids, measuring the absorbance of an aliquot of steroid solution added to previously decomposed sodium borohydride against a reference solution prepared by reducing a similar aliquot with the reagent. In this way, interference from other types of steroids and from many formulation components which may be present in the sample is obviated. According to Görög (5), steroids with the 4-en-3-one function are reduced in 15 min. at room temperature using sodium borohydride in methanol; however, those with the 1,4-dien-3-one chromophore require heating with the reagent and sodium hydroxide for 1 hr.

Since the reactivity of the borohydrides increases with the covalent character of the counter ion (6), we investigated the effect of substituting lithium borohydride for the sodium salt in an attempt to find more convenient reaction conditions for 1,4-dien-3-ketosteroids. Experiments with some representative 4-en-3-one, 1,4-dien-3-one, and 4,6-dien-3-one steroids indicated that all of these steroids can be determined by the differential UV method with a reaction time of 10 min. at room temperature, using lithium borohydride in tetrahydrofuran for reduction.

The procedure is as follows: Transfer 1-ml. portions of a well-stirred, 10-mg./ml. suspension of lithium borohydride in tetrahydrofuran to each of two 25-ml. volumetric flasks. Destroy the reducing agent in one flask by adding 5 ml. of methanol and 5 drops of concentrated hydrochloric acid. Transfer 1-ml. portions of a tetrahydrofuran solution of the conjugated 3-ketosteroid under test, containing about 300 mcg./ml., to each flask, mix, and let stand 10 min. with occasional agitation. Destroy excess borohydride in the second flask with 5 ml. of methanol and 5 drops of acid; then dilute both solutions to volume with methanol. Determine the absorbance of the solution from the first flask in a 1-cm. cell at its wavelength of maximum absorbance, using the other solution in the reference cell.

Table I provides a summary of results obtained with this procedure on some authentic samples of steroids. The differential UV spectra of the 4-en-3-one and 1,4-dien-3-one steroids were closely similar to the spectra

of the steroids determined in methanol against a solvent blank. Reduction of chlormadinone acetate with borohydride leaves the 4,6-diene conjugated system intact, providing an absorption band at 246 nm. ($a = 49.5$ l./g.cm.). However, differential determination of chlormadinone acetate at its 284-nm. maximum shows no interference, because the reduced species has no absorbance in this area of the spectrum.

The reaction medium was changed to tetrahydrofuran because lithium borohydride reacts vigorously with methanol. The necessity for changing solvent, however, introduced another variable factor in the comparison of the borohydride counter ions. An experiment was conducted with hydrocortisone as the test compound, substituting sodium borohydride for the lithium salt in the proposed procedure. The differential absorbance value found was less than 25% that obtained using lithium borohydride or by use of Görög's (5) conditions, sodium borohydride in methanol.

- (1) M. Legrand, V. Delaroff, and R. Smolik, *J. Pharm. Pharmacol.*, **10**, 683(1958).
- (2) R. A. Bastow, *ibid.*, **19**, 41(1967).
- (3) S. Görög and É. Csizér, *Acta Chim. Acad. Sci. Hung.*, **65**, 41(1970).
- (4) M. H. Penner, D. C. Tsilifonis, and L. Chafetz, *J. Pharm. Sci.*, **60**, 1388(1971).
- (5) S. Görög, *ibid.*, **57**, 1737(1968).
- (6) E. Schenker, in "Newer Methods of Preparative Organic Chemistry," vol. IV, W. Foerst, Ed., Verlag Chemie GMBH, Weinheim/Bergstrasse, West Germany, 1968, p. 199.

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Received September 15, 1971.

Accepted for publication October 21, 1971.

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BOOKS

REVIEWS

Fundamentals of Biochemical Pharmacology. Edited by Z. M. BACQ, R. CUPEK, R. PAOLETTI, and J. RENSON. Pergamon Press, Ltd., Headington Hill Hall, Oxford OX3 OBW, England, 1971. xiv + 659 pp. 18 × 26 cm. Price \$27.00.

The aim of this work is described by the editors as the provision of a textbook emphasizing the biochemical roles and sites of action of drugs and aimed at nonpharmacologists wishing to enter this field. To achieve this aim, the editors have called upon the services of some 50 experts to contribute individual chapters or essays. The editors have set themselves a difficult task at which, I fear, they have not entirely succeeded. The major fault in the book lies in a general lack of cohesiveness between the various contributions; the result is that the book resembles a patchwork of essays with rather minimal interconnections. This detracts from its proposed value as a text unless supplemented by appropriate bridging and coordinating material.

The first section of the book consists of a number of necessarily cursory surveys of experimental techniques that are utilized, not exclusively, in biochemical pharmacology. These individual discussions will probably be of value only to workers thoroughly unfamiliar with a given technique. A second section deals with sub-cellular structures and includes short but good discussions of bacterial cell walls, mitochondria, and lysosomes. A regrettable omission is the absence of any discussion of the structural and molecular

basis of mammalian cell membranes, although membrane transport does receive some discussion in a later section. The remainder of the book deals with neuropharmacology, chemotherapy, drug metabolism, and, very briefly, pharmacogenetics and comparative pharmacology. These sections are the most successful of the book and contain a number of very useful discussions. Neuropharmacology and drug-receptor kinetics and interactions are nicely treated; in particular, the discussion of adrenergic transmission provides a very thorough and readable introduction to the subject. Similarly successful are the various sections dealing with the modification and modulation of the various nucleic acid pathways (including a brief discussion of hormone action) and the newcomer to the field will find that these chapters, with the appended references, will provide a quite useful introduction to the biochemical basis of chemotherapy. The section on drug metabolism, although short, is very clearly written and will also provide a valuable introduction to this important area.

In general, the book is clearly written and respectably printed although some of the drawings could be clearer. It should be of general value to undergraduate and graduate students in pharmacology and biochemical pharmacology in providing a rapid survey of the biochemical basis of drug action and should be of substantial value to the nonbiochemically oriented scientist who wishes to acquaint himself with this area.

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