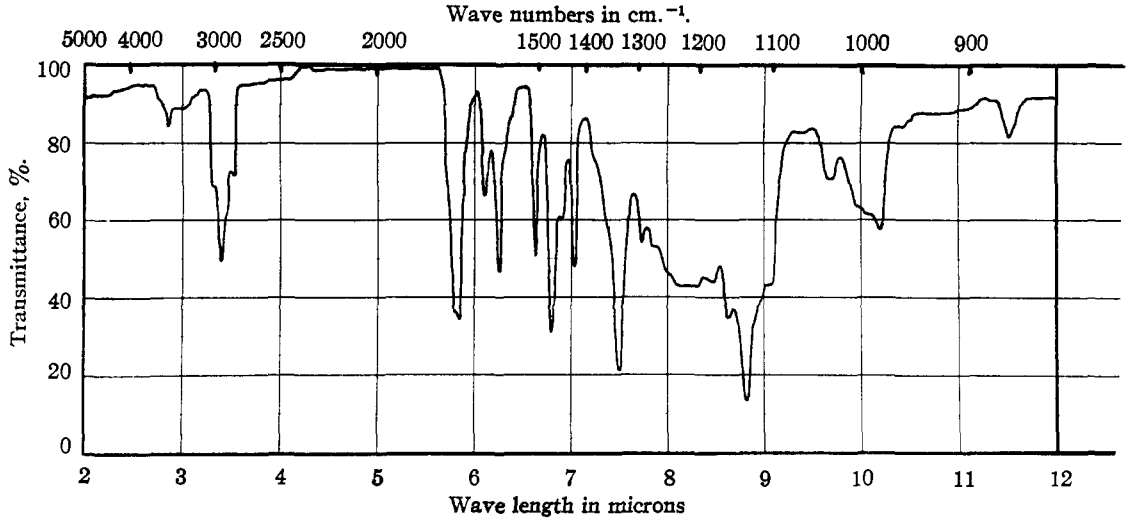


Fig. 1.—Infrared spectrum of reserpine from *Rauwolfia heterophylla* (chloroform solution, 0.1 mm. cell).

"chalchupines" has been corroborated[7] in a study of *R. heterophylla* from Colombia ("piniquepinique") and several pharmacological reports of crude extracts have appeared.[8]

Through the courtesy of Messrs. Mario and Edgar Wunderlich of Guatemala City, we have obtained some authentic *R. heterophylla* from that country while similar material from Mexico was collected by one of us near Oaxaca and identified botanically by Prof. M. Martinez. Chromatography of the benzene-soluble portion of the defatted alcoholic extract of the roots yielded two crystalline alkaloids. The earlier eluted one (m.p. 175–176°, $[\alpha]^{25}D$ −200° ($CHCl_3$), $\lambda_{max.}^{EtOH}$ 292 (3.99), 310 mμ (4.09), $\lambda_{max.}^{CHCl_3}$ 5.62 and 7.93 μ; *Anal.*[9] $C_{19}H_{14}NO_4(OCH_3)_3$ found: C, 63.93; H, 5.66; N, 3.45; methoxyl, 22.12; neut. equiv. ($HClO_4$), 402, Rast mol. wt., 420) was shown to be *l-narcotine* by direct comparison with an authentic specimen of this opium alkaloid kindly supplied by Dr. G. Moersch of Parke, Davis & Company.

The second alkaloid proved to be the widely sought-after *reserpine* (m.p. 262–263°, $[\alpha]^{25}D$ −115° ($CHCl_3$), $\lambda_{max.}^{EtOH}$ 268 mμ (4.15) shoulder at 288–297 mμ (3.95), infrared spectrum in Fig. 1) as demonstrated by direct comparison of the free base and the nitrate with material isolated from the Indian *R. serpentina* and generously furnished by Dr. M. W. Klohs of Riker Laboratories, Inc., and Dr. O. Wintersteiner of the Squibb Institute. We have been able to arrive at a satisfactory empirical formula[4,10] $C_{27}H_{22}N_2O_3(OCH_3)_6$ (Found:[9] C, 65.25; H, 6.42; N, 4.54; methoxyl, 29.83; Rast mol. wt., 619) and if it is assumed that both infrared carbonyl bands at 5.78 and 5.84 μ are due to ester groupings (one of them a methyl ester), then all nine oxygen atoms in reserpine are accounted for. Whether a biogenetic significance can be attributed to the occurrence of both narcotine and reserpine in the same plant must await the structure elucidation of the latter alkaloid. It is noteworthy that the Latin American *R. heterophylla* is the only *Rauwolfia* species other than the Indian *R. serpentina* from which reserpine has so far been isolated.

We are indebted to the Rockefeller Foundation for funds which made possible the plant collections.

JOINT CONTRIBUTION FROM THE
SAMUEL C. HOOKER LABORATORY — CARL DJERASSI
DEPARTMENT OF CHEMISTRY — MARVIN GORMAN[11]
WAYNE UNIVERSITY — A. L. NUSSBAUM[12]
DETROIT 1, MICHIGAN, AND
INSTITUTO DE QUIMICA — JESÚS REYNOSO
UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
TACUBA, D.F.

RECEIVED OCTOBER 14, 1953

(7) R. Paris and R. Mendoza D., *Bull. sci. pharmacol.*, **48**, 146 (1941).

(8) *Cf.* Raymond-Hamet, *Compt. rend.*, **209**, 384 (1939).

(9) Analyses by Mr. J. F. Alicino, Metuchen, N. J.

(10) NOTE ADDED IN PROOF.—Since submission of this paper, three pertinent articles on reserpine have appeared. Our empirical formula is in agreement with that proposed by A. Furlenmeier, *et al.* (*Experientia*, **9**, 331 (1953)) and by N. Neuss, *et al.* (THIS JOURNAL, **75**, 4879 (1953)) but not with that suggested by M. W. Klohs, *et al.* (*ibid.*, **75**, 4867 (1953)). We have confirmed the isolation of trimethylgallic acid from the saponification of reserpine as reported by these three groups.

(11) Pfizer Predoctorate Research Fellow, 1953–1954.

(12) U. S. Public Health Service Predoctorate Research Fellow, 1952–1954.

A REVERSIBLE REACTION OF BOVINE SERUM ALBUMIN

Sir:

We wish to report a reaction of bovine serum albumin (BSA) which has been discovered by a calorimetric procedure.[1] Lowering the *p*H of a BSA solution (ionic strength 0.1 *M*, chloride ion concentration 0.05 *M*) from 4.5 to 3.4 initiates a reaction which absorbs 3,100 cal. per mole of BSA at 25°. The heat absorption follows first order kinetics with high accuracy to more than 90% completion, with a half-time of approximately 2.5 min. The reaction is shown to be completely reversible by the observation that raising the *p*H from 3.4 to 4.5 results in a heat *evolution* of the same magnitude (to within 4%), also following first order kinetics with a half-time of 2.9 min. These heat effects are completely distinguished from the instantaneous heat changes which accompany changes

(1) A. Buzzell and J. M. Sturtevant, THIS JOURNAL, **73**, 2454 (1951).

in the ionization state of the protein when the *p*H is altered. The equilibrium in the reaction is dependent on the *p*H; present indications are that the equilibrium is well over on the side of higher heat content at *p*H 3.0, and on the side of lower heat content at *p*H 4.8.

The nature of the reaction is of course not shown by the calorimetric data. The fact that the kinetics of the heat changes is first order in both directions indicates that the reaction is probably not a dissociation. This conclusion is substantiated by preliminary measurements of osmotic pressures. The most reasonable possibilities would appear to be a swelling of the molecule, or an unfolding of polypeptide chains, resulting from electrostatic repulsions between the positive charges introduced by lowering the *p*H. It remains to be seen whether the reaction is related to other peculiarities in the behavior of serum albumin, such as the increase in the viscosity[2] of albumin solutions with decrease in *p*H, the decrease in sedimentation constant[3] below *p*H 4, and the electrophoretic heterogeneity[4] in the region of the isoelectric point.

An apparently similar reaction has been observed with trypsin in 0.1 *M* chloride solutions in the *p*H range 2.5 to 1.4. In this system the maximum heat change is 8,000 cal. per mole (1 mole ≈ 20,000 g.), and the reaction rates are comparable to those observed with serum albumin. Although trypsin has no enzymatic activity in this *p*H range, its activity is fully regained at neutral *p*H. Measurements of osmotic pressures indicate no change in the state of aggregation of trypsin in the *p*H range of interest. Attempts to find a reaction of similar type in the case of insulin have given negative results.

In our experiments to date we have used Armour crystallized BSA and Worthington salt-free crystalline trypsin. We plan to carry out extensive calorimetric measurements with highly purified proteins, and to parallel these measurements by detailed physical characterization in an effort to establish the nature of the reaction.

We are indebted to the Rockefeller Foundation and to the National Science Foundation for financial assistance in this investigation.

(2) S. Björnholm, E. Barbu and M. Macheboeuf, *Bull. soc. chim. biol.*, **34**, 1083 (1952).

(3) T. Svedberg and B. Sjögren, THIS JOURNAL, **52**, 2855 (1930).

(4) R. A. Alberty, *J. Phys. Coll. Chem.*, **53**, 114 (1949).

DEPARTMENT OF COLLOID SCIENCE
UNIVERSITY OF CAMBRIDGE HERBERT GUTFREUND
CAMBRIDGE, ENGLAND

CONTRIBUTION NO. 1189
STERLING CHEMISTRY LABORATORY
YALE UNIVERSITY JULIAN M. STURTEVANT
NEW HAVEN, CONNECTICUT

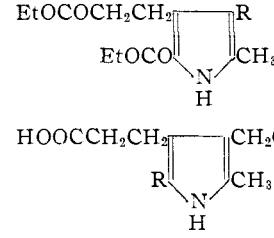
RECEIVED SEPTEMBER 19, 1953

THE SYNTHESIS OF HEMOPYRROLE-DICARBOXYLIC ACID[1]

Sir:

Of the key pyrroles related to the uroporphyrins, cryptopyrrole-dicarboxylic acid has already been synthesized.[2] We now report the synthesis of the second of these, hemopyrrole-dicarboxylic acid (IIb).

The pyrrole (Ia)[2] was converted into the glyoxylic ester (Ib), m.p. 78.5–79°, with ethyl cyanoformate and HCl. Hydrogenation in acetic acid–sulfuric acid with palladium black (ref. 3) then gave (Ic) (50%), m.p. 63–64°; *Anal.* Calcd. for $C_{17}H_{25}O_6N$: C, 60.16; H, 7.43; N, 4.13. Found: C, 59.98; H, 7.26; N, 4.26. The tricarboxylic acid (IIa), m.p. 155–156° (dec.), was obtained by alkaline hydrolysis, and partially decarboxylated with water at 100° to hemopyrrole-dicarboxylic acid (IIb), m.p. 150–150.5° (dec.); *Anal.* Calcd. for $C_{10}H_{13}O_4N$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.78; H, 6.26; N, 6.82.



(Ia) R = H
(Ib) R = COCOOEt
(Ic) R = CH_2COOEt

(IIa) R = COOH
(IIb) R = H

These structures were confirmed by decarboxylating (IIa) at 200° to hemopyrrole-carboxylic acid, m.p. 128–129° (lit. 130–131°[4]), giving the chocolate-brown methyl ester-picrate, m.p. 118.5–120° (lit. 121–122°[4]).

(3) K. Kindler, W. Metzendorf and Dschi-yin-Kwok, *Ber.*, **76**, 308 (1943).

(4) H. Fischer and H. Orth, "Chemie des Pyrrols," Akademische Verlag, Leipzig, 1934, Band I, p. 282 ff.

DIVISION OF PURE CHEMISTRY S. F. MACDONALD
NATIONAL RESEARCH COUNCIL OF CANADA
OTTAWA, CANADA R. J. STEDMAN

RECEIVED SEPTEMBER 30, 1953

IN VITRO INCORPORATION OF LEUCINE INTO THE PROTEINS OF *MICROCOCCUS LYSODEIKTICUS*

Sir:

Lysis of *Micrococcus lysodeikticus* cells with lysozyme under certain conditions has yielded a particulate system, distinct from intact cells, which carries out the incorporation of leucine into protein. Work in this laboratory on baterial amino acid incorporation has been briefly referred to.[1,2] While this work was in progress, Gale and Folkes[3] have reported in a note similar experiments with fragmented cells of *Staphylococcus aureus*. These represent the first cases of amino acid incorporation by bacterial cell fractions.

Resting cells of *M. lysodeikticus* rapidly incorporate leucine into protein. When thoroughly washed cells are treated with lysozyme, all incorporating activity is lost. Attempts to reactivate this lysate by the addition of numerous metabolites were unsuccessful. However, the addition of sucrose during lysis resulted in maintenance of a considerable portion of the activity. The effect of the sucrose was not significantly changed by using

(1) Issued as N.R.C. 3085.

(2) S. F. MacDonald, *J. Chem. Soc.*, 4176, 4184 (1952).

(1) H. Borsook, "Advances in Protein Chemistry," Academic Press, Inc., New York, N. Y., 1953.

(2) H. Borsook, *Fortschr. Chem. org. Naturstoffe*, Springer Verlag, Vienna, Austria, 1952, pp. 310–311.

(3) E. F. Gale and J. P. Folkes, *Biochem. J.*, **55**, xi (1953).