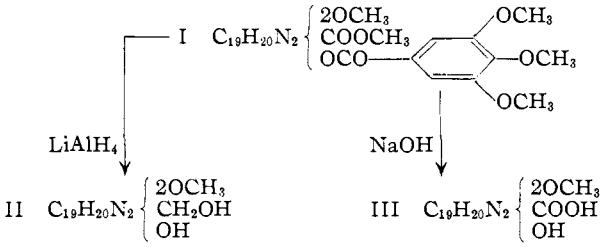
dried for analysis three hours at room t. and 0.05 mm.: Calcd. for $C_{22}H_{23}N_2O_5 \cdot HCl \cdot CH_3OH$: C, 58.90; H, 7.09; Cl, 7.56. Found: C, 58.84; H, 6.85; Cl, 7.53. *Mol. wt.* Calcd. 468.93; found 454 ± 20 (electrometric titration in 66% dimethylformamide, *pK'*a 6.2 and 8.2). Reduction of Reserpin using lithium aluminum hydride in tetrahydrofuran also yielded two products. One was identified as 3,4,5-trimethoxybenzyl alcohol. The *p*-nitrobenzoate was prepared and shown to be identical with the derivative obtained from an authentic sample of the alcohol (infrared, m.p. and mixed m.p.). The apparently new derivative melts at 143°. Calcd. for $C_{17}H_{17}O_7N$: C, 58.79; H, 4.93; N, 4.03. Found: C, 58.78; H, 5.02; N, 4.01. The second fragment for which we propose the name Reserpic alcohol crystallizes with one mole of water, m.p. 216–217° (dec.). Calcd. for $C_{22}H_{30}O_4N_2 \cdot H_2O$: C, 65.32; H, 7.97; N, 6.93; OCH_3 (2), 15.33; act. H, 5 moles. Found: C, 65.12, 65.40; H, 7.99, 7.99; N, 6.67; OCH_3, 15.07; act. H, 4.82 moles. *Mol. wt.* Calcd. 404; found, 409 ± 10 (electrometric titration, *pK'*a 7.7).

As a result of these data the following partial structures are suggested for Reserpin (I), Reserpic alcohol (II) and Reserpic acid (III)



A. Stoll and A. Hofmann[3] have just reported the isolation of a new alkaloid from *Rauwolfia serpentina* Benth. which they named Sarpagin and formulated as a $C_{19}H_{22}O_2N_2$ compound. There appears to be a relationship between Reserpic alcohol and Sarpagin. This relationship is substantiated by the properties of the demethylation product of Reserpic alcohol (with HBr in AcOH). Like Sarpagin this substance reduces Fehling solution and silver nitrate in ammonia solution in the cold. Furthermore, it gives a blue color with Folin–Ciocalteu reagent[4] (positive phenol test).

The $C_{19}H_{22}N_2$ nucleus of Reserpin is possibly a substituted indole alkaloid of Yohimbine-like type and our spectral data are in very good agreement with such a formulation. The infrared spectrum of Reserpin in chloroform solution has a free NH band at 2.87 μ within 0.01 μ of similar bands in indole, 5,6-dimethoxyindole, 2,3-dimethyl-5,6-dimethoxyindole and tetrahydroalstonilin.[5] Two carbonyl bands have wave lengths of 5.79 μ and 5.82 μ in the spectrum (0.01 to 0.02 μ greater separation when analytically resolved). Methyl cyclohexanecarboxylate and methyl 3,4,5-trimethoxybenzoate have bands at 5.78 μ and 5.82 μ, respectively. Comparison of the above-mentioned models with Reserpin in the 6–7 μ region suggests the possibility that one of the two methoxyls in Reserpin is at the 6 position of the indole moiety. A summation of the ultraviolet absorption of 2,3-dimethyl-5,6-dimethoxyindole and methyl 3,4,5-trimethoxybenzoate in a mole per mole ratio resulted in a spectrum which contained all the significant features of the Reserpin spectrum. The computed spectrum possessed three maxima, a shoulder and two minima, at 214 mμ (log ε = 4.73), 267 mμ (log ε = 4.14), 298 mμ (log ε = 4.04), 225 mμ, 246 mμ (log ε = 3.98) and 286 mμ (log ε = 3.96), respectively. The 214 mμ band is due to the methyl 3,4,5-trimethoxybenzoate chromophore. This chromophore is also responsible for a large part of the absorption at 267 mμ. The substituted indole chromophore is the main contributor to the 298 mμ band and also the shoulder at *ca.* 225 mμ. The slight discrepancy between the sum of the absorption of the two compounds and Reserpin can be accounted for by the unknown position of the second methoxyl group in the indole ring of Reserpin.

Acknowledgment.—We would like to thank Dr. R. B. Woodward for helpful suggestions during the course of this investigation. We gratefully acknowledge the aid rendered by the following: Dr. H. A. Rose, X-ray data; Mr. E. H. Stuart, Isolation of the Oleoresin fraction; Messrs. W. L. Brown, G. M. Maciak and H. L. Hunter, elementary analyses and group determinations; Miss M. Hoffman, infrared spectra and Mr. D. O. Woolf, electrometric titration.

LILLY RESEARCH LABORATORIES
INDIANAPOLIS 6, INDIANA

NORBERT NEUSS
HAROLD E. BOAZ
JAMES W. FORBES

RECEIVED SEPTEMBER 8, 1953

(3) A. Stoll and A. Hofmann, *Helv. Chim. Acta*, **36**, 1143 (1953).

(4) O. Folin and V. Ciocalteu, *J. Biol. Chem.*, **73**, 629 (1927).

(5) We would like to thank Dr. R. C. Elderfield, of the University of Michigan, for the sample of Tetrahydroalstonilin.

THE C-25 ISOMERISM OF SARSASAPOGENIN AND SMILAGENIN

Sir:

It has been assumed that sarsasapogenin[1] and smilagenin, yamogenin and diosgenin, and other so-called "normal" and "iso" (22a and 22b) sapogenins differ in their steric arrangement at C-22.[2] This assumption was supported by such observations as sarsasapogenin and smilagenin giving the same "pseudo" compound[3] and the same dihydrogenin.[4]

We have established a difference between pseudosarsasapogenin (I), $[\alpha]^{20}D$ +12°, m.p. 167–169°[5] (*Anal.* Found: C, 77.86; H, 10.32), and psuedosmilagenin (II), $[\alpha]^{20}D$ +24°, m.p. 158–161° (*Anal.* Found: C, 78.06; H, 10.58), and between dihydrosarsasapogenin (III), $[\alpha]^{20}D$ −4°, m.p. 165–167° (*Anal.* Found: C, 77.62; H, 11.16), and dihydrosmilagenin (IV), $[\alpha]^{20}D$ +3°, m.p. 164–

(1) We are indebted to Dr. Monroe E. Wall, Eastern Regional Research Laboratory, United States Department of Agriculture, for a generous supply of this material.

(2) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publishing Corp., New York, N. Y., 1949, p. 578 ff.

(3) R. E. Marker, E. Rohrmann and E. M. Jones, THIS JOURNAL, **62**, 648 (1940).

(4) R. E. Marker and E. Rohrmann, *ibid.*, **61**, 846 (1939).

(5) Rotations, unless otherwise noted, were taken in chloroform solution. All melting points reported were taken on the Kofler block and are uncorrected.

166° (*Anal.* Found: C, 77.69; H, 11.31). The di-3,5-dinitrobenzoate of I melts at 192–194°, $[\alpha]^{20}D$ +29° (*Anal.* Found: C, 60.80; H, 6.00; N, 6.73), and of II melts at 176–178°, $[\alpha]^{20}D$ +19° (*Anal.* Found: C, 61.17; H, 6.42; N, 6.97). We prepared the diacetate of III, m.p. 68–69°, $[\alpha]^{20}D$ −3° (*Anal.* Found: C, 74.02; H, 9.86), and of IV, m.p. 91–93°, $[\alpha]^{20}D$ −1° (*Anal.* Found: C, 73.90; H, 10.05). The dibenzoate of III melts at 96–98°, $[\alpha]^{20}D$ +3° (*Anal.* Found: C, 78.37; H, 8.85), and that of IV at 138–140°, $[\alpha]^{20}D$ + 2° (*Anal.* Found: C, 78,33; H, 8.58). Moreover, the acid-catalyzed rearrangement of pseudosarsasapogenin and of pseudosmilagenin led quantitatively to sarsasapogenin and to smilagenin, respectively.

We were able to isolate from the mixture resulting from the oxidation of pseudosarsasapogenin the dextrorotatory α-methylglutaric acid,[6] $[\alpha]^{20}D$ +18° (EtOH), m.p. 78.5–81° (*Anal.* Found: C, 49.40; H, 6.76). From pseudosmilagenin we obtained the enantiomorphic levorotatory α-methylglutaric acid, $[\alpha]^{20}D$ −20° (EtOH), m.p. 78.5–81° (*Anal.* Found: C, 49.79; H, 7.03). A mixture of these isomers melted at 66–68°. Comparison of the infrared absorption spectra of these compounds with an authentic sample of α-methylglutaric acid showed all three to be identical.

Finally, the selective tosylation of the primary hydroxyl group at C-26 and subsequent reduction with lithium aluminum hydride converted dihydrosarsasapogenin and dihydrosmilagenin to the identical 16,22-epoxycoprostan-3β-ol, $[\alpha]^{20}D$ −4°, m.p. 137–139°; *Anal.* Found: C, 80.39; H, 11.73. The benzoate melts at 138–140°, $[\alpha]^{20}D$ +1°; *Anal.* Found: C, 80.57; H, 10.27. Thus it appears established that the isomerism of sarsasapogenin and smilagenin rests in the asymmetry at C-25.

(6) (a) R. E. Marker, D. L. Turner, R. B. Wagner, P. R. Ulshafer, H. M. Crooks and E. L. Wittle, THIS JOURNAL, **63**, 779 (1941); (b) Berner and Leonardsen, *Ann.*, **538**, 1 (1939).

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
BETHESDA 14, MARYLAND

IRVING SCHEER
ROBERT B. KOSTIC
ERICH MOSETTIG

ON THE MECHANISM OF THE ENZYMATIC SYNTHESIS OF GLUTATHIONE

Sir:

The synthesis of GSH[1] from L-γ-glutamyl-L-cysteine, glycine and ATP by a pigeon liver enzyme has been shown to involve the liberation of an equivalent amount of P_i.[2] Recently the enzyme which catalyzes the same reaction has been isolated from brewer's yeast autolysate and purified 1,500-fold.[3] Using the purified yeast enzyme, the quantitative changes in nucleotides as well as the P_i liberated during GSH synthesis have now been determined. As is shown in Table I, GSH synthesis is accompanied by the liberation from ATP of an equivalent amount of both P_i and ADP. The over-all reaction may hence be formulated as

$$\text{L-}\gamma\text{-glutamyl-L-cysteine} + \text{glycine} + \text{ATP} \longrightarrow \text{GSH} + \text{ADP} + P_i \quad (1)$$

In the course of attempts to elucidate the detailed mechanism of GSH synthesis, the exchange of phosphate residues between ADP and ATP was tested in the same system. P^{32}-ATP was isolated from a rabbit which had been injected with P^{32}-orthophosphate. Incubation of the radioactive ATP with AMP and muscle myokinase yielded P^{32}-ADP which was purified on a Dowex-1 column,[4] and isolated as the barium salt. As shown in Table II, low concentration of the yeast enzyme effects a transfer of the phosphate from ATP to ADP.

TABLE I

BALANCE STUDY OF GSH SYNTHESIS

The reaction mixture, 20.0 ml., contained 0.01 *M* tris buffer, 0.005 *M* KCN, 0.004 *M* $MgSO_4$, 0.005 *M* C^{14}-glycine, 0.002 *M* glutamylcysteine, 0.015 mg. of yeast enzyme per ml. and 0.4% bovine serum albumin. Incubation was 60 min. at 37°, *p*H 8.5. GSH was determined by isotopic assay,[5] P_i by the method of Gomori,[6] and the nucleotides by Siekevitz and Potter's modification,[7] of the Dowex-1 technique of Cohn and Carter.[4] The results are expressed in μM per ml. of reaction mixture.

	GSH Synthesized	P_i	AMP	ADP	ATP
Initial	0	0	0.04	0.45	1.18
Final	0.48	0.49	.04	.89	0.71
Δ	+ .48	+ .49	0	+ .44	− .47

TABLE II

PHOSPHATE EXCHANGE BETWEEN RADIOACTIVE ADP AND ATP

The reaction mixture, 3.0 ml., contained 0.01 *M* tris buffer, 0.005 *M* KCN, 0.004 $MgSO_4$, 3.12×10^{-3} *M* P^{32}-ADP, 2.53×10^{-3} *M* ATP, and 0.1% bovine serum albumin. Incubation was 60 min. at 37°, *p*H 8.5. Nucleotides were separated and assayed by the Dowex-1 technique.[4]

Enzyme, mg./ml.	ADP, c.p.m./μM.	ATP, c.p.m./μM.	Exchange, %
...	2370	39	..
0.001	2280	400	29
.003	1530	1280	92
.030	1440	1380	99

Since AMP was not formed and since concentrations of ATP and ADP remained unchanged under these conditions it may be concluded that the observed exchange is not due to myokinase action. The exchange data may be interpreted as evidence for the reversible phosphorylation of the enzyme.

$$\text{Enzyme} + \text{ATP} \longrightarrow \text{Enzyme-P} + \text{ADP} \quad (2)$$

$$\text{Enzyme-P} + P^{32}\text{-ADP} \longrightarrow \text{Enzyme} + P^{32}\text{-ATP} \quad (3)$$

In view of the highly purified state of the enzyme, it appears highly probable that the exchange of phosphate between ATP and ADP is effected by the same enzyme which is responsible for GSH synthesis. Furthermore reactions 2 and 3 are consistent with the known powerful inhibition of GSH

(1) The following abbreviations have been used: GSH, glutathione; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; Tris, tris-(hydroxymethyl) aminomethane.

(2) J. E. Snoke, S. Yanari and K. Bloch, *J. Biol. Chem.*, **201**, 573 (1953).

(3) J. E. Snoke, unpublished results.

(4) W. E. Cohn and C. E. Carter, THIS JOURNAL, **72**, 4273 (1950).

(5) R. B. Johnston and K. Bloch, *J. Biol. Chem.*, **188**, 221 (1951).

(6) G. Gomori, *J. Lab. Clin. Med.*, **27**, 955 (1940).

(7) P. Siekevitz and V. R. Potter, *J. Biol. Chem.*, **200**, 188 (1953).