TABLE I

ELECTROPHORETIC FRACTIONATION OF AVIDIN NA

Component	Mobility[a]	N/P ratio	Ultraviolet absorption: E	E_p[b]	E_N[b]	Activity %[c]	Soly. in water
Main	− 2.0	100	Max. 281 Max. 290 Min. 250		130	91	Sol.
Minor	−15.4	2.8[d]	Max. 258 Min. 232	8,100	1360	4	Sol.
Unfractionated material		14	Max. 260 Min. 243	10,000	310	100	Insol.

[a] Mobilities × 10^5 cm.^{2} volt^{-1} at 0° and *p*H 8.9 in 0.2 *M* K_2HPO_4. Similar mobilities were observed for avidin NA and its biotin complex at approximately 0.5 and 1% protein concentration, respectively. The mobilities vary with the solvent; in veronal-chloride buffers at 0.1 ionic strength, mobilities similar to those reported by Woolley and Longsworth[2] have been observed. [b] E_p calculated according to Vischer and Chargaff (*J. Biol. Chem.*, **176**, 703, 715 (1949)), E_N correspondingly on the basis of the N content of the preparations. [c] In terms of % of the unfractionated material, 1 mg. of which inactivated 7.4 γ biotin. [d] Upon fractionation of the biotin complex of avidin NA the minor component obtained had N/P ratio of 2.1, which indicates that it is largely nucleic acid.

moiety. This is evident not only from the electrophoretic fractionations, but also from the finding that a readily water-soluble biotin-binding fraction can be isolated from egg white free from nucleic acid, yet almost as active as avidin NA. This albumin (avidin A) is probably identical with the protein component of avidin NA.

The question whether avidin is naturally associated with a nucleic acid, or whether the complex is formed only during the isolation, regardless of the method employed, is under investigation. Nucleoproteins appear not to have been previously recognized and isolated from egg white. Their possible embryological significance, particularly in association with avidin, invites speculation.

WESTERN REGIONAL RESEARCH LABORATORY
ALBANY, CALIFORNIA H. L. FRAENKEL-CONRAT
BUREAU OF AGR. AND INDUSTRIAL CHEMISTRY
AGRICULTURAL RESEARCH ADMINISTRATION W. H. WARD
UNITED STATES DEPARTMENT N. S. SNELL
OF AGRICULTURE E. D. DUCAY

RECEIVED JUNE 26, 1950

STROPHANTHUS AGLYCONES

Sir:

In a search for sources of sarmentogenin and other 11-oxygenated steroidal aglycones, we have examined a number of different species of *Strophanthus*. Because of the current interest in the *Strophanthus* species, we should like to report our findings at this time. We employed a modification of the procedure of Katz[1] for the isolation of the steroidal aglycones. With seeds of *S. sarmentosus*, *S. hispidus*, *S. kombe* and *S. eminii* our data agree in general with those published previously by other investigators.

(1) A. Katz, *Helv. Chim. Acta*, **31**, 993–1004 (1948).

Sarmentogenin (0.15%; m. p. 270–274°; mixed with authentic sample, no m. p. depression) and a new aglycone, sarverogenin (0.10%), recently described by Reichstein and his associates,[2] were obtained from seeds of *S. courmontii* (sample number 50R344). Strophanthidol (0.15% was isolated from seeds of *S. mortehanii* (50R1241) and from seeds of *S. arnoldianus* (9R7837) in very small yields. No sarmentogenin was isolated from the seeds of either *S. thollonii* (9R9113 and 9R9114) or *S. preussii* (50R581 and 50R582). The samples, however, were small and further work on larger samples is in progress.

Isolation studies with seeds of *S. congoensis* (5OR580) yielded a major Legal-positive steroid (1.02%) and two minor Legal-positive steroids (0.03% and 0.01%). The major steroid has been characterized and it appears to be sarverogenin; m. p. 219–222°C. (micro-block); *Anal.* Found: C, 65.84, 65.91; H, 7.48, 7.27; $[\alpha]^{23}D$ +49.5° (*c* = 1.0; methanol); $\lambda_{max.}$ 2160 Å. (ethanol), *E*% 433. The molecular weight by the ebullioscopic method in methanol was 437 ± 14. An infrared spectra comparison of our steroid with sarverogenin showed the two to be almost identical, exhibiting bands at 5.78, 5.88, 6.12 and 2.97 μ as solids in Nujol mull. A mixture of the two compounds gave no melting point depression and each gave the same sequence of color changes when a few crystals were contacted with sulfuric acid.[3] Attempts to prepare the acetyl derivative failed to give a crystalline product. However, a crystalline benzoate was prepared: m. p. 187–191° (micro-block); *Anal.* Found: C, 70.61; H, 6.69; $[\alpha]^{23}D$ +30.7° ± 1° (*c* = 1.301; acetone). These properties and the sequence of colors produced with this derivative in sulfuric acid are in agreement with sarverogenin dibenzoate.[2]

In addition to seeds, other parts of plants (branchlets with leaves, stem bark, root bark, stem wood, root wood, inner shells of fruits and awns of seeds) were examined for steroid content. From numerous samples of *S. sarmentosus*, *S. barteri*, *S. gratus*, *S. hispidus* and *S. preussii* minute quantities of Legal-positive reacting fractions were obtained in only a few cases. Apparently the glycoside resides almost exclusively in the seeds, at least in the species which were investigated. A detailed report of our work will be presented at a later date.

The samples investigated in this work were collected and identified by B. A. Krukoff. The botanical information and the places of deposit of the herbarium specimens have been reported in

(2) von Euw, Katz, Schmutz and Reichstein, "Festschrift Prof. Paul Casparis" S. 178 (Zürich, 1949); Buzas, von Euw and Reichstein, *Helv. Chim. Acta*, **33**, 465 (1950). Since submission of this manuscript, von Euw and Reichstein reported the isolation of Saveroside, sarmentocymarin and sarmentogenin from *S. courmontii*; *Helv. chim. acta*, **33**, 1006 (1950).

(3) We are indebted to Dr. A. Katz, presently at The Experimental Biology and Medical Institute, National Institute of Health, Bethesda, Maryland, for carrying out these comparisons.

part by Krukoff and Letouzey,[4] the remainder will be published later.

(4) B. A. Krukoff and R. Letouzey, *Rev. de Bot. Appl.* 329-350, Mars-Avril, 1950.

JOHN W. ROTHROCK
E. E. HOWE
KLAUS FLOREY
MAX TISHLER
RESEARCH LABORATORIES
MERCK & CO., INC.
RAHWAY, NEW JERSEY

RECEIVED JUNE 26, 1950

ABSORPTION SPECTRA OF SULFURIC ACID CHROMOGENS OBTAINED FROM ADRENAL STEROIDS AND RELATED COMPOUNDS

Sir:

The observation that certain adrenocortical steroids when treated with concentrated sulfuric acid furnish colored solutions, has been recorded by several authors.[1,2] However, no data have been published concerning the absorption spectra of these chromogens. In order to establish the possible analytical value of such spectral data, the absorption curves of the chromogens obtained from the six active adrenal steroids and 8 other related compounds have been determined. The procedure used was as follows: 3 ml. of concentrated sulfuric acid (reagent grade) was added to 70 to 90 micrograms of the dry steroid in a test-tube. The tube was stoppered and allowed to stand at room temperature for two hours. The optical density of the solution, from 220 to 600 mμ, was then read in a Beckman spectrophotometer, using concentrated sulfuric acid as a blank.

Table I summarizes the results obtained with the fourteen steroids studied. The curves obtained were all different with respect to shape and position of the absorption maxima. Acetates and free compounds gave identical curves. One of the important features of the procedure is the small amount of material required for analysis. The absorption spectra of these chromogens have already proven of great value when used in conjunction with the paper chromatographic method of analysis for adrenal steroids,[4] in establishing the identity of compounds isolated from biological sources.

TABLE I

Compounds[3]	Absorption maxima, mμ
17-Hydroxycorticosterone	280, 395, 475
17-Hydroxy-11-dehydrocorticosterone	280, 343, 415
Corticosterone	285, 330, 373, 455
11-Dehydrocorticosterone	280, 355, 415
17-Hydroxy-11-desoxycorticosterone	285, 535
11-Desoxycorticosterone	285, 370, 440
allo-Pregnane-3(β),11(β),17(α)-21-tetrol-20-one	330, 415, 510
allo-Pregnane-3(β),17(α),21-triol-11,20, dione	333, 410
allo-Pregnane-3(β),17(α),21-triol-20-one	315, 410
Pregnane-17(α);21-diol-3,11,20-trione	270, 340, 415
3-Hydroxy-11-keto-etiocholanic acid	320, 405
3,9-Epoxy-11-keto-etiocholanic acid	290, 405
Δ⁴-Androstene-3,11,17-trione	280
Androstane-3,11,17-trione	No maxima

(1) Wintersteiner and Pfiffner, *J. Biol. Chem.*, **116**, 291 (1936).

(2) Reichstein and Shoppee, "Vitamins and Hormones," **1**, 345 (1943).

(3) Generously donated by Drs. T. F. Gallagher, P. L. Julian, E. C. Kendall, C. D. Kochakian, M. H. Kuizenga, H. L. Mason, E. Oppenheimer, G. Pincus, T. Reichstein and C. R. Scholz.

(4) Zaffaroni, Burton and Keutmann, *Science*, **111**, 6 (1950).

(5) National Cancer Institute Postdoctoral Fellow.

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF ROCHESTER
SCHOOL OF MEDICINE AND DENTISTRY
ROCHESTER, NEW YORK ALEJANDRO ZAFFARONI[5]

RECEIVED JUNE 27, 1950

THE TOTAL SYNTHESIS OF A 5-PHENYL PENICILLIN: METHYL 5-PHENYL-(2-CARBOMETHOXYETHYL)-PENICILLINATE

Sir:

By the use of a new method we have synthesized a 5-phenyl penicillin which has the complete structure of the natural penicillins, including the fused thiazolidine-β-lactam ring system with all of the correctly situated substituents, and in addition possesses a 5-phenyl group.

Interaction of methyl 2-phenyl-5,5-dimethyl-2-thiazoline-4-carboxylate[1] (II), succinimidoacetyl chloride (I) and triethylamine yielded 4-carbomethoxy - 5,5 - dimethyl - 2 - phenyl - α - succinimido-2-thiazolidineacetic acid β-lactam (III) in 13% yield, m. p. 186.8–187.4° (cor.), obtained as the cyclohexane solvate. *Anal.* Calcd. for $C_{19}H_{20}N_2O_5S \cdot {}^1/_2C_6H_{12}$: C, 61.38; H, 6.09; N, 6.51. Found: C, 61.17; H, 6.12; N, 6.42. In tetrachloroethane solution the infrared absorption spectrum showed bands at 5.65, 5.72 and 5.84 μ, assignable to the β-lactam carbonyl, the ester carbonyl and the succinimido ring, respectively. Oxidation of III with potassium permanganate in acetic acid–dioxane–water mixture by a procedure identical with that used in preparing penicillin methyl ester sulfone[2] afforded an 82% yield of the sulfone (IV), m. p. 230.0° (cor.) with decomposition. *Anal.* Calcd. for $C_{19}H_{20}N_2O_7S$: C, 54.32; H, 4.79; N, 6.67. Found: C, 54.10; H, 4.90; N, 6.73. After alkaline hydrolysis of III, N-phenacylsuccinamic acid was isolated as the 2,4-dinitrophenylhydrazone, m. p. 186.4–187.2°, undepressed upon admixture with an authentic sample (m. p. 186.4–187.3°) prepared in a similar manner from N-phenacylsuccinimide.[3] *Anal.* Calcd. for $C_{18}H_{17}N_5O_7$: C, 52.05; H, 4.13; N, 16.86. Found: C, 51.69; H, 4.24; N, 16.72.

Selective basic hydrolysis of III followed by esterification with diazomethane yielded methyl 5-

(1) This thiazoline is readily obtained through the reaction of ethyl benzimidate hydrochloride with penicillamine methyl ester Sheehan and Buhle, THIS JOURNAL, in preparation.

(2) H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 177. This book also provides a good review of previous attempts to synthesize penicillin and related β-lactams.

(3) Scheiber and Reckleben, *Ber.*, **46**, 2413 (1913).