was added slowly, with stirring, and the reaction mixture refluxed for two hours. This method can undoubtedly be used to advantage to prepare related compounds that are unsaturated in other than the Δ^5 position. Ring F of spirostanols and spirostenols is not cleaved by $LiAlH_4$ in the absence of hydrogen chloride gas.

Some of the compounds that we have prepared by this method are[3]: reduction of Δ^5-22-isospirosten-3β-ol (diosgenin) yielded Δ^5-furostene-3β,26-diol (dihydrodiosgenin) (I), m.p. 158–160°[4], $[\alpha]^{20}D$ −35° $CHCl_3$ (*Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.77; H, 10.84). Sandoval, *et al.*[5] reported an optical rotation of $[\alpha]^{20}D$ −33° for $\Delta^{5,20(22)}$-furostadiene-3β,26-diol (ψ-diosgenin). Acetylation of (I) at room temperature yielded Δ^5-furostene-3β,26-diol 3,26-diacetate (dihydrodiosgenin diacetate) (II), m.p. 115–117°, $[\alpha]^{20}D$ −39° $CHCl_3$ (*Anal.* Calcd. for $C_{31}H_{48}O_5$: C, 74.36; H, 9.66. Found: C, 74.45; H, 9.73).

Reduction of 22-isoallospirostan-3β-ol (tigogenin) (III) yielded allofurostane-3β,26-diol (dihydrotigogenin) (IV), m.p. 163–166° (lit. m.p. 167–170°[2]), $[\alpha]^{20}D$ −4° $CHCl_3$ (*Anal.* Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.41; H, 10.92). Acetylation of (IV) at room temperature yielded allofurostane-3β,26-diol 3,26-diacetate (dihydrotigogenin diacetate) (V), m.p. 116–117° (lit. m.p. 114–116°[2]), $[\alpha]^{20}D$ −15 $CHCl_3$ (*Anal.* Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 74.15; H, 10.04).

Reduction of spirostan-3β-ol (sarsasapogenin) yielded furostane-3β,26-diol (dihydrosarsasapogenin) (VI), m.p. 157–160° (lit. m.p. 165°)[6], $[\alpha]^{20}D$ −2° $CHCl_3$ (*Anal.* Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.45; H, 11.05). Benzoylation of VI at 95° for one hour yielded a crystalline product, furostane-3β,26-diol-3,26-dibenzoate (dihydrosarsasapogenin dibenzoate) (VII), m.p. 95–97°, (*Anal.* Calcd. for $C_{41}H_{54}O_5$: C, 78.55; H, 8.68. Found: C, 78.37; H, 8.51).

It will be noted that sapogenins having the "normal" and "iso" configuration at carbon 22 and the "normal" and "allo" configuration at carbon 5 are cleaved by $LiAlH_4$ in the presence of hydrogen chloride gas but not in its absence. The stability of rings E and F of the steroidal sapogenins to cleavage by $LiAlH_4$ (alkaline) is further confirmed by the work of Djerassi, *et al.*[7] In contrast to the stability of the sapogenins, tomatidine, a steroidal secondary amine,[8] which has yielded Δ^{16}-allopregnen-3β-ol-20-one[9] by what we consider to be typical steroidal sapogenin reactions, is cleaved by $LiAlH_4$ (alkaline) to yield a diol compound, dihydrotomatidine.[8] The structural relationship between tomatidine and steroidal sapogenins will be reported in a future publication.

BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY
AGRICULTURAL RESEARCH CENTER HARRY M. DOUKAS
BELTSVILLE, MARYLAND THOMAS D. FONTAINE

RECEIVED OCTOBER 8, 1951

(3) We are indebted to M. E. Wall, Eastern Regional Research Laboratory, Wyndmoor, Pennsylvania, for supplying the spirostanols and spirostenols used in this work and for determining optical rotations.

(4) Melting points were taken in capillary tubes in an oil bath and are corrected.

(5) A. Sandoval, J. Romo, G. Rosenkranz, St. Kaufmann and C. Djerassi, THIS JOURNAL, **73**, 3820 (1951).

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

(7) C. Djerassi, H. Martinez and G. Rosenkranz, *J. Org. Chem.*, **16**, 1278 (1931).

(8) T. D. Fontaine, J. S. Ard and R. M. Ma, THIS JOURNAL, **73**, 878 (1951).

(9) Y. Sato, A. Kats and E. Mosettig, *ibid.*, **73**, 880 (1951).

ELECTRODIALYSIS OF SHEEP ADRENOCORTICOTROPIC (ACTH) PROTEIN PREPARATIONS[1]

Sir:

Adrenocorticotropic hormone (ACTH) protein preparations isolated from sheep and pig pituitary glands by the methods of Li, *et al.* and Sayers, *et al.*,[2] appear to be homogeneous by sedimentation, electrophoresis and solubility studies. Despite this apparent homogeneity, there is now considerable evidence to indicate that the biological activity associated with the protein does not involve the whole protein molecule.[3]

We wish to report the results of electrodialysis experiments on protein preparations[2a] in which the protein is separated into two main fractions. A three-cell electrodialysis apparatus was used.[4] The center cell was separated from the anode by a goldbeater's skin and from the cathode by a vegetable parchment membrane. In a typical experiment, a 0.4% ACTH protein solution (*p*H 3.6) was introduced into the center cell; the anode cell contained 0.5% acetic acid (25 ml.) while the cathode contained distilled water (25 ml.) which

TABLE I

DISTRIBUTION OF NITROGEN AND BIOLOGICAL ACTIVITY IN VARIOUS FRACTIONS OBTAINED BY ELECTRODIALYSIS OF SHEEP ACTH PROTEIN PREPARATIONS

Expt.[a]	Fraction	Nitrogen, mg.	Nitrogen, %	Bioassay[b] ACTH equiv. mg.	Bioassay[b] ACTH equiv. %
	Starting prepn. (L 2011A)	12.6	100	56 (12)[c]	100
I	Center	9.6	76	0 (9)	0
	Combined cathodes	1.9	15	55 (15)	98
	Anode	0.1	0.5	...	...
	Starting prepn. (L 2220A)	5.3	100	85 (15)	100
II	Center	4.1	77	0.6 (6)	<1
	Combined cathodes	1.1	21	53 (20)	62
	Anode	0.0	0	...	...

[a] Electrodialysis was carried out for 5 hours. [b] As measured by the ascorbic acid depletion method of Sayers, *et al.*[5] [c] Number of rats used in parentheses.

(1) Assisted by grants from the National Institutes of Health, United States Public Health Service, the Armour Laboratories, Merck and Company, and Eli Lilly Laboratories.

(2) (a) C. H. Li, H. M. Evans and M. E. Simpson, *J. Biol. Chem.*, **149**, 413 (1943). (b) G. Sayers, A. White and C. N. H. Long, *ibid.*, **149**, 425 (1943).

(3) (a) C. H. Li, *Trans. Macy Conf. on Metabolic Aspects of Convalescence*, **17**, 114 (1948); (b) N. G. Brink, M. A. P. Meisinger and K. Folkers, THIS JOURNAL, **72**, 1040 (1950); (c) J. B. Lesh, J. D. Fisher, I. M. Bunding, J. J. Kocsis, L. S. Walaszek, W. F. White and E. E. Hays, *Science*, **112**, 43 (1950); (d) R. W. Payne, M. S. Rahen and E. B. Astwood, *J. Biol. Chem.*, **187**, 719 (1950). (e) B. Cortis-Jones, A. C. Crooke, A. A. Hewly, P. Morris and C. J. O. R. Morris, *Biochem. J.*, **46**, 173 (1950).

(4) A modification of the apparatus of H. Theorell and Å. Åkesson (*Arkiv. Kemi Mineral. Geol.*, **16A**, No. 8, 1943), designed and kindly made available to us by Professor C. A. Knight.

was changed at hourly intervals during the experiment. A current of 10–20 milliamperes was maintained by suitable variation of the applied voltage (500–2000 volts).

In two typical experiments (Table I) 15–20% of the total nitrogen passed through the parchment membrane into the cathode cell. When the cathode fraction was assayed for ACTH potency by the ascorbic acid depletion method of Sayers, *et al.*,[5] it was found to contain almost all the hormone activity. The residue (77% of the total nitrogen) in the center compartment contained less than 1% of the original activity.

Incubation with pepsin[3a] in 0.01 *N* hydrochloric acid solution for 24 hours at 37° or heating a 1% solution in 0.2 *N* hydrochloric acid in a boiling water-bath[6] for one hour did not lead to inactivation of the cathode fraction. In preliminary ultracentrifugal studies on the cathode fraction, only one component with an S_{20} value of approximately 1.0 was detected.

(5) M. Sayers, G. Sayers and L. A. Woodbury, *Endocrinology*, **42**, 379 (1948).

(6) C. H. Li, THIS JOURNAL, **73**, 4146 (1951).

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA
GEORGE P. HESS
J. IEUAN HARRIS
FREDERICK H. CARPENTER
CHOH HAO LI

RECEIVED OCTOBER 22, 1951

THE PATHWAY OF INOSITOL FERMENTATION IN AEROBACTER AEROGENES[1]

Sir:

Previous work from this Laboratory[2] has indicated that *myo*-inositol[3] is metabolized by a pathway not involving conversion to glucose and degradation according to the Embden–Meyerhoff scheme. Further information has been obtained by the use of suspensions of *A. aerogenes* grown with strong aeration in a mineral medium containing glucose or *myo*-inositol as the sole source of carbon. Such cells are unable to split formic acid[4] or to produce acetoin. Thus, the fermentation of glucose, studied in a conventional Warburg apparatus in bicarbonate buffer in an atmosphere of 5% CO_2 and 95% N_2, resulted in the formation of acid, but not of CO_2 or H_2. In a typical experiment 0.32 mole of ethanol, 0.79 mole of lactate and 1.23 equivalents of other acids were formed per mole of glucose. When the phosphoclastic attack on pyruvate was inhibited by 0.0016 *M* arsenite, 1.95 mole of lactate were formed.

myo-Inositol, 2-keto-*myo*-inositol, L-1-keto-*myo*-inositol, and L-1,2-diketo-*myo*-inositol were fermented by cells grown on *myo*-inositol, but not by cells grown on glucose. All four substrates yielded acid and CO_2 but no H_2. 2-Keto-*myo*-inositol and L-1,2-diketo-*myo*-inositol were fermented two to three times as fast as *myo*-inositol or L-1-keto-*myo*-inositol. The fermentation of *myo*-inositol produced 0.68 mole of CO_2, 0.72 mole of ethanol and 1.54 equivalents of acid per mole. In the presence of arsenite, 0.72 mole of CO_2, 0.72 mole of ethanol, 0.68 mole of lactate, and 0.56 equivalent of unidentified acids were formed. These results show clearly that the CO_2 and the ethanol formed from *myo*-inositol are not derived from pyruvate.

On the basis of these and earlier experiments the following pathway of *myo*-inositol degradation may be tentatively suggested: *myo*-inositol $-$ $2H\rightarrow$2-keto-*myo*-inositol $-$ $2H\rightarrow$L-1,2-diketo-*myo*-inositol $-$ $2H\rightarrow CO_2$ + acetate + pyruvate + $6H\rightarrow$ CO_2 + ethanol + lactate.

These end-products account for 70% of the *myo*-inositol. Since the first steps in this scheme are dehydrogenations, the greater rate of fermentation found for 2-keto-*myo*-inositol and L-1,2-diketo-*myo*-inositol may be explained by their ability to act as hydrogen acceptors.

Similar pathways in which dehydrogenations precede cleavage of the carbon chain may not be restricted to inositol. The recent demonstration[5] that fermentation of glucose by *Leuconostoc mesenteroides* yields equimolar quantities of CO_2, ethanol and lactate, and that the rate of fermentation may be increased by hydrogen acceptors indicates that a similar scheme may be the major glycolytic pathway in that organism.

(5) R. D. DeMoss, R. C. Bard and I. C. Gunsalus, *J. Bact.*, **62**, 499 (1951).

DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY
HARVARD MEDICAL SCHOOL
BOSTON 15, MASS.
BORIS MAGASANIK

RECEIVED NOVEMBER 7, 1951

CRYSTALLIZATION OF A DERIVATIVE OF PROTOGEN-B

Sir:

The protozoan *Tetrahymena geleii* needs an unidentified fraction present in liver and other natural materials for growth.[1,2] The name "protogen" has been used to designate the substance or substances which are responsible for this growth effect. Concentrates with "protogen" activity have been shown to be needed for the growth of an unidentified *Corynebacterium*[3] and to have activity corresponding to the "acetate factor"[4,5] and the "pyruvate oxidation factor."[6]

Reed, *et al.*,[7] have reported the crystallization of a compound designated α-lipoic acid which appears to have biological properties similar to those of protogen.

The protogen activity of a papain digest of a water-insoluble fraction of liver was not extractable by organic solvents. However, after autoclaving with 3.3 *N* sodium hydroxide and acidifying with hydrochloric acid, the activity could be extracted with chloroform. Counter-current extraction by the method of Craig[8] using several solvent systems

(1) Supported by the William F. Milton Fund.

(2) B. Magasanik, Am. Chem. Soc., Abst. of Papers, 119th Meeting, 20C (1951).

(3) Formerly *meso*-inositol. For the nomenclature of inositol derivatives see H. G. Fletcher, Jr., L. Anderson, and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951).

(4) J. L. Stokes, *J. Bact.*, **57**, 147 (1949).

(1) G. W. Kidder and V. Dewey, *Arch. Biochem.*, **8**, 293 (1945).

(2) E. L. R. Stokstad, *et al.*, *ibid.*, **20**, 75 (1949).

(3) E. L. R. Stokstad, *et al.*, *Proc. Soc. Expll. Biol. Med.*, **74**, 571 (1950).

(4) E. E. Snell and H. P. Broquist, *Arch. Biochem.*, **23**, 326 (1949).

(5) B. M. Guirard, *et al.*, *ibid.*, **9**, 381 (1946).

(6) D. J. O'Kane and I. C. Gunsalus, *J. Bact.*, **56**, 499 (1948).

(7) L. J. Reed, *et al.*, *Science*, **114**, 93 (1951); *J. Biol. Chem.*, **192**, 851, 859 (1951).

(8) L. C. Craig and O. Post, *Anal. Chem.*, **21**, 500 (1949).