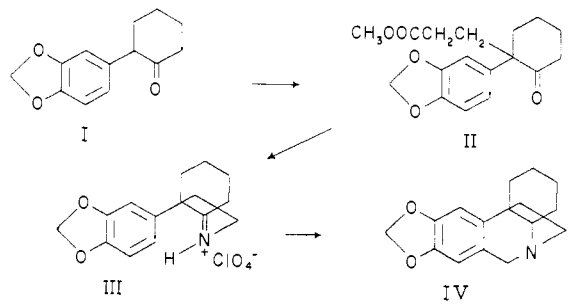
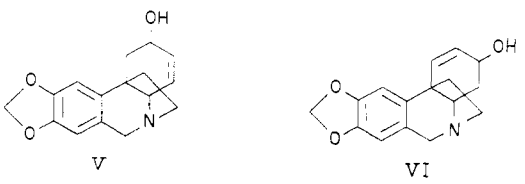
(m.p. 209–209.5°; found: C, 70.89; H, 6.31; N, 5.35). Dihydroöxocrinine (m.p. 157–159°; found: C, 70.96; H, 6.17; N, 5.12; $\lambda_{max}^{CHCl_3}$ 5.84; λ_{max}^{EtOH} 237 mμ (3.56), 294 mμ (3.72)), obtained by the catalytic hydrogenation of oxocrinine, formed a dibenzylidene derivative (m.p. 125°; found: C, 80.31; H, 5.88). Under mild Wolff–Kishner conditions,[4] dihydroöxocrinine gave *l*-crinane (5,10b-ethano-8,9-methylenedioxy-1,2,3,4,5,6,10b,10c-octahydrophenanthridine), (b.p. 130° (1 μ); $[\alpha]^{24}D$ −12.7°; found: C, 74.57; H, 7.63; N, 5.37); picrate (m.p. 206–207°; found: C, 54.40; H, 4.52; N, 11.51). *l*-Crinane also was obtained from the catalytic hydrogenation-hydrogenolysis of crinine.

dl-Crinane was synthesized in the following manner. 2-(3,4-Methylenedioxyphenyl)-cyclohexanone (m.p. 93–94°; found: C, 71.33; H, 6.55) was obtained from 5-(3,4-methylenedioxyphenyl)-4-nitrocyclohexene[5] by the Nef reaction.[6] Cyanoethylation of I and subsequent methanolysis of the nitrile gave II (m.p. 88–89°; found: C, 67.15; H, 6.51). The action of nitrous acid on the hydrazone hydrazide[7] of II gave 2,3,4,5,6,7-hexahydro-3a-(3,4-methylenedioxyphenyl)-indole which was isolated as the hydroperchlorate (III), (m.p. 227–229°; found: C, 52.51; H, 5.27; Cl, 10.14). Catalytic hydrogenation of III and Pictet–Spengler cyclization of the resultant base afforded *dl*-crinane (IV), (m.p. 97–99°; found: C, 74.69; H, 7.27; N, 5.41); picrate (m.p. 218–220°; found: C, 54.49; H, 4.35; N, 11.60). The infrared spectra (liquid film) of the *l*-crinane and the synthetic crinane were superimposable, as were the spectra of the natural and synthetic picrates in chloroform solution.



Crinine must be represented by V or VI. A 3-hydroxy-Δ^{4-4a} structure is unlikely since dihydrocrinine (pK_a 8.70) is only slightly more basic than crinine (pK_a 7.95).[8] The stability of dihydrooxocrinine to base and its methohydroxide to the Hofmann degradation favors structure V.



Consistent with the morphine-like skeleton of these alkaloids, several exhibit significant analgesic activity.[9]

TABLE I

PRELIMINARY RESULTS ON ANALGESIC ACTIVITY OF AMARYLLIDACEAE ALKALOIDS AND THEIR DERIVATIVES

Alkaloid	ED_{50}	LD_{50}	Duration min.
Buphanidrine	6.2	8.9	106
dl-Crinane	20	40	...
Crinine	45	~100	...
Galanthamine	2.7	11.3	141
Lycoramine	21.3	89	133
Morphine	2.1	576	129

(4) M. Gates and G. Tschudi, THIS JOURNAL, **78**, 1380 (1956).

(5) L. H. Mason and W. C. Wildman, *ibid.*, **76**, 6194 (1954).

(6) W. C. Wildman and R. B. Wildman, *J. Org. Chem.*, **17**, 581 (1952).

(7) W. E. Bachmann and E. J. Fornefeld, THIS JOURNAL, **73**, 51 (1951).

(8) V. Prelog and O. Hafliger, *Helv. Chim. Acta*, **32**, 1851 (1949).

(9) I am indebted to Dr. Nathan B. Eddy, National Institute of Arthritis and Metabolic Diseases, for the pharmacological data.

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RECEIVED JULY 16, 1956

STUDIES ON ADRENOCORTICOTROPIN. XIII. THE ISOLATION OF TWO HIGHLY ACTIVE BOVINE TYPES

Sir:

By means of chromatography on the carboxylic type ion exchange resin XE-97[1] we have separated two highly potent types of adrenocorticotropin from beef pituitary extracts. As shown in Fig. 1, the two active types (A_1 and A_2) emerge from the column as clearly separated retarded peaks.[2] The distinction between the two peaks in terms of hold-up volumes is maintained when the peptide material recovered from each peak is re-run separately through the same column. The salt-free peptide products recovered[3] from the peaks show potencies in the USP test of about 85 units per milligram by the intravenous method. This value is slightly lower than those obtained for the purest porcine[4,5] and sheep[6] preparations, but is several times higher than the best potencies yet obtained for fractions isolated from beef sources.[7]

The starting material used in our columns was an oxycellulose eluate fraction made from crude extracts of beef anterior glands by a process similar to that described by Astwood, *et al.*[8] As noted by others,[7] our oxycellulose eluates from beef extracts have lower potencies than those from pork by a

(1) Rohm and Haas Co., Washington Square, Philadelphia 5, Pa.

(2) Although we had previously (in our work with pork ACTH) numbered our chromatographic peaks in the order of their emergence from the column, we are here adopting the reverse convention used by Dixon and Stack-Dunne in their recent paper (*cf.* reference 10).

(3) For de-salting the column fractions, we have used two methods with equal success: (a) three 45 minute dialyses against 10 volumes of 0.1 *N* acetic acid, or (b) distribution to phenol and back again to water. Our procedure in the latter method is similar to that of Dixon and Stack-Dunne (*cf.* reference 10) except that we use 0.02 *N* acetic acid as the aqueous phase and lyophilize the final solution directly.

(4) W. F. White, THIS JOURNAL, **75**, 503 (1953).

(5) P. H. Bell, *ibid.*, **76**, 5565 (1954).

(6) C. H. Li, I. I. Geschwind, J. S. Dixon, A. L. Levy and J. I. Harris, *J. Biol. Chem.*, **213**, 171 (1955).

(7) W. T. Koch and F. J. Wolf, THIS JOURNAL, **77**, 489 (1955).

(8) E. B. Astwood, M. S. Raben, R. W. Payne and A. B Grady, *ibid.*, **73**, 2969 (1951).

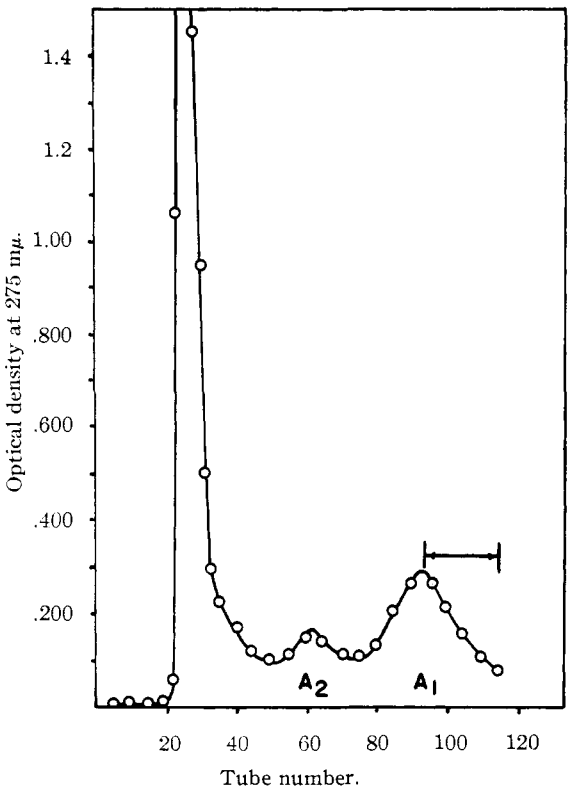

Fig. 1.—Chromatography of a beef oxycellulose eluate ACTH preparation on Amberlite XE-97 resin. The sample (420 mg.) in 25 ml. *p*H 8.85 buffer (0.1 *M* sodium carbonate–bicarbonate) was applied to a column 68 cm. high in a tube 3.4 cm. in diameter. The rate of flow was 22.5 ml./cm.^{2}/hour. The volume collected per tube was 10 ml. Recovery of solids in the active peaks was: A_1, 20 mg.; A_2, 13.5 mg.

factor of one-third to one-fourth. Correspondingly, the weight yields of the purified fractions from our columns are lower for beef than for pork.

In studying the behavior of beef oxycellulose eluate materials on the XE-97 columns, it was found that the *p*H conditions previously used by us for pork fractions[9] were unsuitable. No retarded peaks were observed at *p*H 8.5 and little activity was recovered. However, as the *p*H of the buffer was raised, retarded peaks appeared. Resolution of the peaks and recoveries of activity reached maximum values at *p*H 8.85. Figure 1 shows a curve obtained under the best conditions. The two active peaks appear to correspond to those obtained by Dixon and Stack-Dunne[10] for pork materials.

Preliminary physical and chemical studies have been made on material recovered from the A_1 peak. In order to minimize contamination from the A_2 peak, only the second half of the A_1 peak was used (marked in Fig. 1). The ultraviolet absorption curve of this material was identical with that obtained with corticotropin-A. In 0.1 *N* hydrochloric acid a maximum was obtained at 275 mμ and a minimum at 250 mμ. The best preparations from both species show a 275/250 ratio of 2.2. Paper chromatography of an acid hydrolysate of the bovine product give an amino acid pattern similar to that obtained from corticotropin-A: cystine, threonine and isoleucine are absent, while arginine, histidine, lysine, tyrosine, phenylalanine, methionine, serine, leucine, valine, glycine, glutamic acid, aspartic acid, alanine and proline are present. The presence of tryptophan, suggested by the ultra-violet absorption curve, was confirmed by its appearance on chromatograms of an aminopeptidase digest. An N-terminal determination by the dinitrofluorobenzene method showed only serine,[11] and aminopeptidase gave an amino acid release pattern identical with that obtained with corticotropin-A.[12] Treatment of the bovine product with carboxypeptidase also duplicated the result obtained with corticotropin-A.[13]

Further studies concerning the relationship between the purified bovine product and corticotropin-A are in progress.

(9) W. F. White and W. L. Fierce, THIS JOURNAL, **75**, 245 (1953).

(10) H. B. F. Dixon and M. P. Stack-Dunne, *Biochem. J.*, **61**, 483 (1955).

(11) As in the case of corticotropin-A, the purified beef preparation gave a yield of DNP-serine corresponding to only 15–20% of the amount calculated on the basis of a molecular weight in the range of 5000. The low yield appears to be due to the proximity of the tyrosine residue, since both synthetic seryltyrosine and synthetic seryltyrosylserylmethionylglutamic acid (both peptides courtesy of Dr. K. Hofmann, University of Pittsburgh) also give low yields of DNP-serine.

(12) W. F. White, THIS JOURNAL, **77**, 4691 (1955).

(13) W. F. White, *ibid.*, **75**, 4877 (1953).

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RECEIVED JUNE 8, 1956

DEGRADATION OF GLYCOGEN TO ISOMALTOTRIOSE

Sir:

It has generally been considered that the α-D-(1 → 4) and α-D-(1 → 6) linkages occur in glycogen in a ratio of approximately twelve to one.

Glycogen (130 g., Pfanstiehl) was subjected to acid hydrolysis under conditions which would reduce "reversion" to a negligible amount, and the resulting hydrolyzate was subjected successively to chromatographic separation on a carbon column, on filter paper, and to paper electrophoresis. This resulted in a yield of 300 mg. of amorphous material, $[\alpha]^{23}D$ +128° (*c* 3.5, water). The material was chromatographically homogeneous, moving on paper at a rate slightly slower than panose (4-α-isomalto pyranosyl-D-glucose) but much more rapidly than panose on paper electrophoresis using borate buffer at *p*H 10. A similar fast moving spot has been noted[1] in the hydrolyzate of yeast glycogen but was not further investigated.

A molecular weight determination on the amorphous, fully acetylated reduction product (alditol) of the isolated material indicated a trisaccharide structure. The reduced trisaccharide was subjected to partial hydrolysis and in the hydrolyzate, D-glucose, D-glucitol and isomaltose (or isomaltitol) were identified by paper chromatography and isomaltose and D-glucose by paper electrophoresis. The acetylated hydrolysis products were then sep-

(1) S. Peat, W. J. Whelan and T. E. Edwards, *J. Chem. Soc.*, 355 (1955).