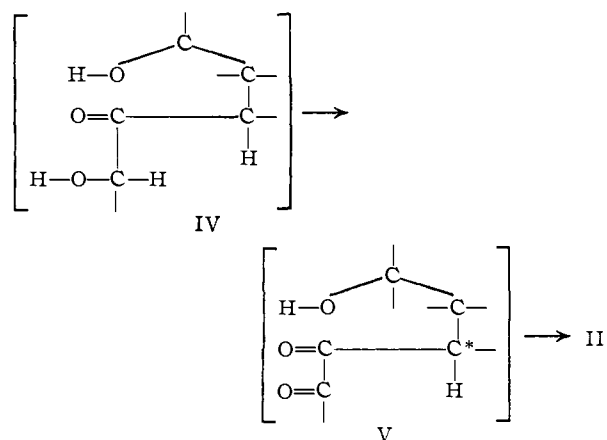


oxidation of cevine and veracevine we regard as resulting from the series I → II → III.

The formation of the 6-membered lactone from cevagenine is explicable on the basis that the α -diketone (V) resulting from oxidation of the α -ketol (IV) epimerizes easily at C* and is converted to the hemiketal (II).



Acknowledgment.—We should like to express our appreciation to Mrs. Mary Fieser and Professor R. B. Woodward for stimulating discussions and suggestions. The work was supported by grants from the National Institutes of Health and Research Corporation.

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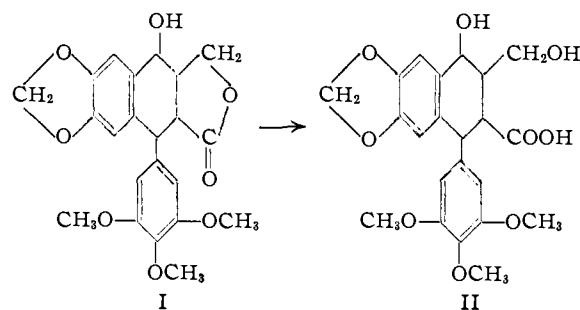
RECEIVED DECEMBER 9, 1953

(5) On leave of absence from the Weizmann Institute of Science, Rehovot, Israel.

SYNTHESIS OF A DL-STEREOISOMER OF PODOPHYLLIC ACID¹

Sir:

Podophyllic acid (II) is the hydrolysis product of the anti-cancer agent, podophyllotoxin (I).



or of its epimer,² picropodophyllin.^{2,3,4} We wish to report the synthesis of a racemic stereoisomer of this acid.

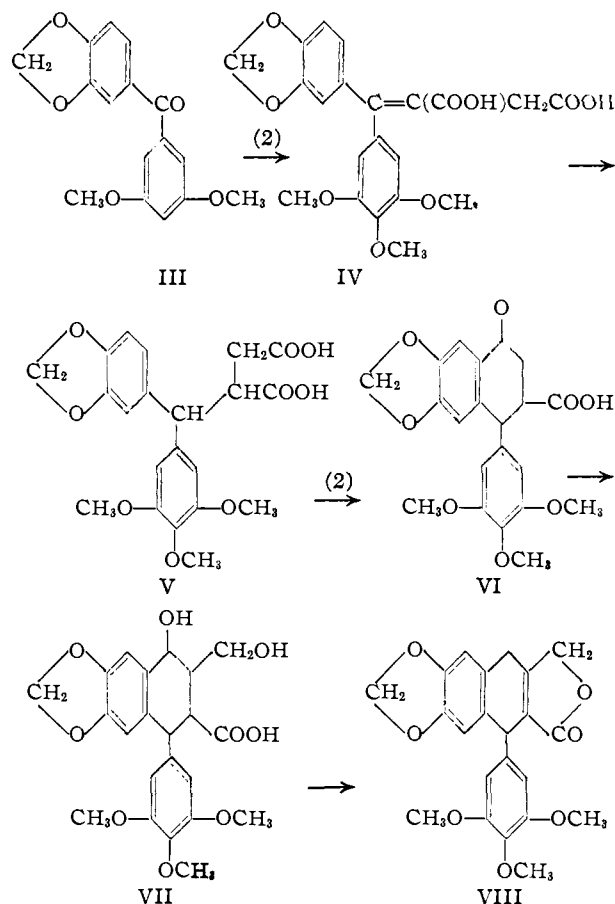
(1) This work has been supported by grants-in-aid from the American Cancer Society upon recommendation of the Committee on Grants of the National Research Council.

(2) J. L. Hartwell and A. W. Schrecker, *THIS JOURNAL*, **73**, 2909 (1951).

(3) W. Borsche and J. Niemann, *Ann.*, **494**, 126 (1932).

(4) W. R. Dustan and T. A. Henry, *J. Chem. Soc.*, **73**, 209 (1898); R. Kürsten, *Arch. Pharm.*, **229**, 220 (1891).

Stobbe condensation of ketone III⁵ with ethyl succinate followed by saponification furnished the unsaturated dibasic acid IV, m.p. 191.4–192.2°. *Anal.* Calcd. for C₂₁H₂₀O₉: C, 60.6; H, 4.8. Found: C, 60.5; H, 4.8. This compound on hydrogenation gave a single product V, m.p. 192.7–



193.2°. *Anal.* Calcd. for C₂₁H₂₂O₉: C, 60.3; H, 5.3. Found: C, 60.2; H, 5.3. The dibasic acid V was treated first with acetyl chloride to form the anhydride (a glass; *Anal.* Calcd. for C₂₁H₂₀O₈: C, 63.0; H, 5.0. Found: C, 62.9; H, 5.1), and then with aluminum chloride in nitrobenzene to form the cyclic keto acid VI, m.p. 221.7–222.5°. *Anal.* Calcd. for C₂₁H₂₀O₈: C, 63.0; H, 5.0. Found: C, 63.0; H, 5.2.⁶ Keto acid VI with ethanol and sulfuric acid gave the corresponding keto ester (m.p. 153–154°. *Anal.* Calcd. for C₂₃H₂₄O₈: C, 64.5; H, 5.6. Found: C, 64.3; H, 5.7), which on condensation with ethyl formate in the presence of sodium hydride followed by sodium borohydride reduction afforded a dihydroxy ester. Saponification yielded the desired product, dihydroxy acid VII, m.p. 228.6–229.6°. *Anal.* Calcd. for C₂₂H₂₄O₉: C, 61.1; H, 5.6. Found: C, 61.1; H, 5.7. The reported melting point of "natural" podophyl-

(5) E. Späth, F. Wessely and E. Nadler, *Ber.*, **66**, 125 (1933); W. J. Gensler and C. M. Samour, *THIS JOURNAL*, **73**, 5555 (1951).

(6) G. N. Walker, *ibid.*, **75**, 3390 (1953), reported m.p. 220–223° for keto acid VI prepared by a different route. We find that the melting point of the methyl ester is 180.7–181.2°. *Anal.* Calcd. for C₂₃H₂₄O₈: C, 63.8; H, 5.4. Found: C, 63.5; H, 5.3. The reported melting point is 173–175°.

lic acid trihydrate is 163–165° (dec.)^{2,3} at which temperature picropodophyllin (I) is reformed. The melting point of the anhydrous acid³ is not recorded.

The present synthesis is unambiguous with the exception of one step. Cyclization of dibasic acid V could lead to a compound isomeric with VI in which the trimethoxyphenyl ring is part of the tetralin system and the methylenedioxyphenyl ring is a substituent. However, this possibility was excluded by conversion of the dihydroxy acid VII (treatment with boiling water or boiling 10% aqueous sulfuric acid) to *dl*- β -apopicrodophyllin the structure of which is accepted⁷ as VIII. The identity of the synthetic apo compound [m.p. 215–216°; *Anal.* Calcd. for C₂₂H₂₀O₇: C, 66.7; H, 5.1. Found: C, 66.7; H, 5.3] was demonstrated by the infrared absorption curve, which was indistinguishable from that of "natural" β -apopicrodophyllin, and by the ultraviolet absorption curve, which showed a maximum and a minimum of the same intensities and wave lengths as those reported^{7,8} for the "natural" material.

Further work on the relation of the synthetic dihydroxy acid VII to podophyllin acid, and to picropodophyllin and podophyllotoxin is under way.

(7) Cf. A. W. Schrecker and J. L. Hartwell, *THIS JOURNAL*, **74**, 5676 (1952).

(8) N. L. Drake and E. H. Price, *ibid.*, **73**, 201 (1951).

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RECEIVED OCTOBER 5, 1953

HIGH STREPOGENIN POTENCY OF SYNTHETIC OXYTOCIN AND OF PURIFIED VASOPRESSIN

Sir:

The bacterial and animal growth factor strepogenin, which is a peptide-like substance liberated during partial hydrolysis of certain proteins,^{1,2} has previously not been isolated in pure condition. Consequently, considerable doubt and confusion has prevailed about its nature and biological functioning. The application of ion-exchange chromatography under very carefully controlled conditions, and of countercurrent distribution, has now resulted in the isolation from partial hydrolysates of insulin (concd. HCl, 37°, 24 hours)³ of eight peptides, each of which exhibited very high strepogenin activity for *Lactobacillus casei*. These peptides behaved as homogeneous substances in several highly selective fractionation procedures, but no final claim of their purity is being made. The most active one was 780 times as potent as the standard liver extract² and thus gave half-maximal growth at 0.09 γ

(1) D. W. Woolley, *J. Exp. Med.*, **73**, 487 (1941).

(2) H. Sprince and D. W. Woolley, *THIS JOURNAL*, **67**, 1734 (1945).

(3) D. W. Woolley, *J. Biol. Chem.*, **171**, 443 (1947).

per cc. This peptide yielded on hydrolysis cystine, glutamic acid, glycine, serine, valine and the leucines. The other peptides which ranged in potency from 100 to 600 times the liver extract standard each contained these same amino acids with the following modifications. Some contained tyrosine and some lacked one or two of serine, glycine and isoleucine.

The amino acid composition of these peptides was being investigated when the synthesis of oxytocin was announced by du Vigneaud, *et al.*⁴ The similarity in amino acid composition of our peptides to oxytocin was readily seen. Through the kindness of Dr. du Vigneaud and co-workers samples of synthetic oxytocin and related peptides were obtained. Assays showed that oxytocin was 300 times as active as the liver extract standard. It was, thus, far more potent than any protein digest,² and approached the activity of our best isolated peptide. The fact that the oxytocin was synthetic showed clearly that a polypeptide could have high strepogenin activity. So long as isolated compounds were the only ones with high potency it could be argued that the strepogenin activity resided in some constituent other than those generally recognized to occur in proteins. Such a belief is no longer tenable.

The cystine residue of oxytocin was vital to its strepogenin potency. S,S-Dibenzyl oxytocin was practically inactive (potency less than 6), as was also performic-acid-oxidized oxytocin. Several of the smaller synthetic peptides representing sequences in oxytocin or in vasopressin^{4,5,6} were inert also. This was true of L-isoleucyl-L-glutaminyll-L-asparagine, L-phenylalanyl-L-glutaminyll-L-asparagine and L-glutaminyll-L-asparagine. However, some variation in the amino acid composition was compatible with high strepogenin potency, as shown by assay of the structurally related polypeptide vasopressin. A sample of purified arginine-vasopressin (300 pressor units per mg.), free of oxytocin, was kindly provided by Dr. du Vigneaud for this test. It had a strepogenin activity of 150. Clearly, high strepogenin activity for *L. casei* did not reside in a single structure. However, since many polypeptides, even those containing glutamic acid⁷ and cystine have proven to be almost inert, some specificity of structure obviously is involved.

FROM THE ROCKEFELLER
INSTITUTE FOR MEDICAL
RESEARCH, NEW YORK

D. W. WOOLLEY⁸
R. B. MERRIFIELD

RECEIVED DECEMBER 1, 1953

(4) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis and S. Gordon, *THIS JOURNAL*, **75**, 4879 (1953).

(5) V. du Vigneaud, H. C. Lawler and E. A. Popenoe, *ibid.*, **75**, 4880 (1953).

(6) E. A. Popenoe, H. C. Lawler and V. du Vigneaud, *ibid.*, **74**, 3713 (1952).

(7) D. W. Woolley, *J. Biol. Chem.*, **172**, 71 (1948).

(8) With the technical assistance of V. Armbrust.