pyridine/HOAc/H_2O (30:20:6:24) or *n*-BuOH/HOAc/H_2O (4:1:5) solvent systems. Each purified fraction was submitted to N-terminal residue analysis,[7,8] to C-terminal residue analysis,[6] quantitative amino acid analysis,[8] and finally to sequential degradation from the N-terminus by the Edman method.[5,9] The following peptide fragments were identified: Gly.Lys.Pro.Val.Gly.Lys; Lys.Arg.Arg.-Pro.Val; Arg.Try; Ser.Met.Glu.His.Phe; Ser.-Tyr; Ala.Phe.Pro.Leu; Lys(Val,Tyr,Pro,Asp_2Gly,-Glu_2,Ala).Ser.Ala.Glu.NH_2; Lys.(Val,Tyr,Pro,Asp_2,-Gly,Glu_3,Ala_3,Ser,Phe) and Glu.Phe.

Tryptic digests of the hormone (substrate/enzyme = 90/1 (w./w.), *p*H 9–9.5, 40° for 6 hours) were submitted to countercurrent distribution for 37 transfers in the *n*-BuOH/20% HOAc system. The material with a partition coefficient (*K*) of 2 (peptide T2) was isolated and was shown to be homogeneous by N-terminal and amino acid analyses. Sequential degradation[5,9] of this peptide from the N-terminus, together with analysis by the dinitrophenylation method[7,8] gave the structure: Val. Tyr. Pro. Asp (Gly, Glu_4, Ala_3, Asp, Ser, Phe_2, Pro, Leu). Partial acid hydrolysis (3 *M* HCl, 24 hours at 40°) of this material yielded the following peptides: Ala.Glu.Asp; Gly.Glu(Ala_2,Glu,Asp,Ser) and (Val,Tyr,Pro,Asp,Gly,Glu).Ala.

The remainder of the material from the counter current distribution of tryptic digests of bovine corticotropin was isolated and further separated by zone electrophoresis and paper chromatography by means of the techniques employed for the chymotryptic digests. The peptide fragments listed were identified: Ser.(Tyr,Ser,Met,Glu,His,-Phe).Arg; Try.Gly.Lys.Pro.Val.Gly.Lys; Lys.Arg and Arg.Arg.Pro.Val.Lys.

From the above data, an amino acid sequence is proposed for bovine corticotropin

Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.Lys.Pro.Val.
1 2 3 4 5 6 7 8 9 10 11 12 13

Gly.Lys.Lys.Arg.Arg.Pro.Val.Lys.Val.Tyr.Pro.Asp.Gly.
14 15 16 17 18 19 20 21 22 23 24 25 26

NH_2
|

Glu.Ala.Glu.Asp.Ser.Ala.Glu.Ala.Phe.Pro.Leu.Glu.Phe
27 28 29 30 31 32 33 34 35 36 37 38 39

When this structure is compared with the ovine[10] and porcine[11,12,13] corticotropins,[14] it is notable that the amino acid sequences in all three peptide hormones are identical except in the region between amino acid residues 25 and 33, a region rich in acidic amino acids.

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RECEIVED MARCH 20, 1958

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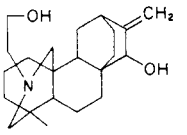
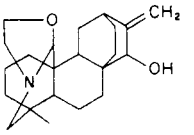
1-METHYL-6-ETHYL-3-AZAPHENANTHRENE, A KEY DEGRADATION PRODUCT OF ATISINE

Sir:

The recent correlation of the aconitum alkaloid atidine[1,2] and the delphinium alkaloid ajaconine[2,3,4] with atisine emphasizes the key position this substance occupies among the diterpene alkaloids of the two genera. Recently structures I and II have been suggested for dihydroatisine[5] and atisine,[6] mainly on the basis of the striking analogy of the chemistry of these substances[7] to that of the garrya alkaloids.[8] Subsequent experimental work has demonstrated the presence of the oxazolidine





moiety[9,10,11] in atisine and isoatisine, the β-aminoethanol group[11,12] in dihydroatisine and the disposition of the D-ring and its substituents.[13] An important piece of evidence bearing on the skeleton of atisine is the structure of the $C_{16}H_{15}N$ base[14] (obtained on selenium dehydrogenation) which contains all but six of the carbon atoms of atisine and relates the heterocyclic ring to the rest of the molecule. We now wish to report the identification of this base as 1-methyl-6-ethyl-3-azaphenanthrene (VII)[15] by an unambiguous synthesis from 7-ethyltetralone-1 (III). This synthesis provides the first evidence fixing the position of the nitrogen atom with respect to the rest of the atisine molecule.

Alkylation of the pyrrolidine enamine of 7-ethyltetralone-1[16] (III) with ethyl α-iodopropionate was effected by the method of Stork[17] to give after

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(15) This structure was suggested by the similarity of the ultraviolet absorption spectrum of VII to that of the $C_{16}H_{15}N$ base[8a] (1-methyl-7-ethyl-3-azaphenanthrene) obtained from the closely related garrya alkaloids.
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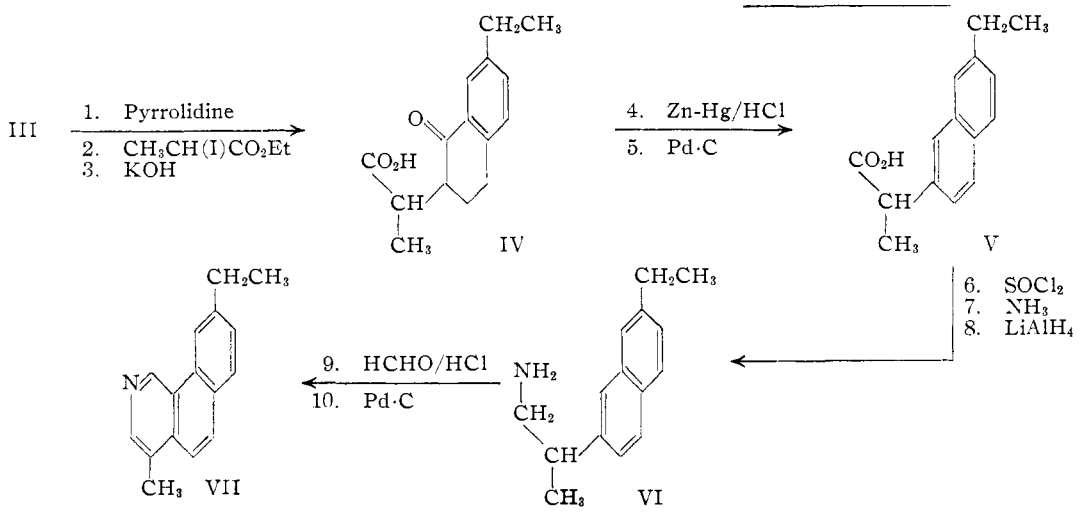
basic hydrolysis a crystalline mixture of acids (IV),[18] various fractions of which showed melting points between 103 and 129°, ν_{max} 1707 cm.$^{-1}$, 1680 cm.$^{-1}$ (Nujol). This material was reduced



by the Clemmensen method to a resinous product.[18] ν_{max} 1712 cm.$^{-1}$ (film) which on dehydrogenation with 10% palladium on carbon[16] at 240–250° furnished the crystalline α-methyl-7-ethyl-2-naphthaleneacetic acid (V),[18] m.p. 109–110°. Treatment of this acid with thionyl chloride followed by anhydrous ammonia in benzene gave the amide,[18] m.p. 105–107°, ν_{max} 1651 (Nujol). This amide was reduced by lithium aluminum hydride to the amine (VI) which was isolated as the hydrochloride,[18] m.p. 208–209°; picrate,[18] m.p. 215–218°. Reaction of the hydrochloride with formalin in 20% aqueous ethanolic hydrochloric acid[19] furnished the cyclization product (tetrahydro VII), also isolated as the crystalline hydrochloride,[18] m.p. 217–221°. Dehydrogenation of the free base was accomplished with 10% palladium on carbon at 225–235° to give 1-methyl-6-ethyl-3-azaphenanthrene (VII)[18] m.p. and mixture m.p. with material[20] from dehydrogenation of atisine, 83.5–85°; picrate,[18] m.p. and mixture m.p., 220–221°; trinitrobenzene adduct,[18] m.p. and mixture m.p. 122.5–123.5°. Infrared and ultraviolet absorption spectra of the two samples of the azaphenanthrene were identical.

(18) Analyses for carbon and hydrogen were satisfactory.

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(20) We wish to express our gratitude to Drs. Jacobs and Craig for providing us with a sample of the $C_{16}H_{15}N$ dehydrogenation product from atisine.

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RECEIVED MARCH 26, 1958

1,2-METHYL SHIFTS IN THE CYCLIZATION OF SQUALENE TO LANOSTEROL[1]

Sir:

For the rearrangement of the carbon skeleton in the cyclization of squalene to lanosterol the alternatives of a single 1,3-methyl shift and two 1,2-methyl shifts have been considered.[2,3] The two mechanisms can be distinguished as follows. All-*trans* squalene composed of the labeled species AA, BB, AB and BA (Fig. 1) was synthesized[4,5] from a mixture of 3-C^{13}, and 4-C^{13} ethyl acetoacetate (65 at. % excess C^{13} in the labeled carbons) and converted enzymatically to lanosterol.[6] The purified lanosterol was oxidized to acetic acid[7] which was converted to ethylene.[8] The relative amounts of $CH_2{=}CH_2$, $C^{13}H_2{=}CH_2$ and $C^{13}H_2{=}C^{13}H_2$ were determined in the mass spectrometer. C^{13}-labeled acetic acid (and hence ethylene) will be derived from $C_{13} + C_{18}$ and $C_{14} + C_{30}$ and diluted by normal acetic acid from other branched portions of lanosterol. Had the labeled carbons initially been 100% C^{13}, the relative amounts of masses 30, 29 and 28 would be 1:4:19 for 1,2-methyl shifts[9] and 0:6:18 for a 1,3-methyl shift. With 65% C^{13} in the labeled position the excess of the labeled ethylenes above normal abundance should be those shown:

For 1,2-Methyl shifts		For 1,3-Methyl shift	
Before dilution	After dilution[10]	Before dilution	After dilution[10]
Excess $C^{13}H_2{=}C^{13}H_2$			
$\frac{0.65 \times 0.65}{24} = 0.117\%$		0	0
1.76			
Excess $C^{13}H_2{=}CH_2$			
$\frac{(2 \times 0.65 \times 0.35 + 4 \times 0.65)^{11}}{24} = 12.7\%$	0.84%	$\frac{6 \times 0.65}{24} = 16.2\%$	1.08%

(1) This work was supported by grants-in-aid from the National Science Foundation, the U. S. Public Health Service, the Life Insurance Medical Research Fund and the Eugene Higgins Trust Fund of Harvard University.

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(9) Since lanosterol has six branched methyl groups which can give rise to acetic acid and since the squalene contained four isotopic species, only one out of twenty-four acetic acid molecules can be doubly labeled.

(10) Non-isotopic acetic acid was added to the acetic acid derived from lanosterol.