fragments: his(ser,glu,gly,thr,phe)[13]; thr(ser,asp, tyr); ser(lys,tyr); leu(asp_2,ser,arg_2,ala,glu,phe); val(glu,try); and leu(met,asp,thr).

Four fragments were isolated from a 2.25 hour tryptic digestion of glucagon: his(ser_3,glu,thr_2,phe, gly,asp,tyr,lys); tyr(leu,asp,ser,arg); arg; and ala-(glu_2,asp_2,phe,val,try,leu,met,thr).

Six peptides were obtained from a 50-hour tryptic digest: his(ser,glu,gly,thr,phe); thr(ser_2,asp, tyr,lys); tyr(leu,asp,ser,arg); arg; ala(glu_2,asp, phe,val,try); and leu(met,asp,thr).

Subtilisin digestion followed by chromatographic resolution gave the following eleven peptides: his(ser,glu); gly(thr,phe); thr.ser; asp(tyr,ser); lys.tyr; leu(asp,ser,arg); arg(ala,glu); asp.phe; val(glu,try); leu.met; and asp.thr.

In a great majority of cases specific enzymatic splits of greater than 80% were obtained and the resulting peptides were isolated in yields exceeding 50%. With every enzyme used, the sum of the resulting fragments was in complete agreement with the empirical formula of glucagon.

The structure of peptide LT-4 was completely elucidated by partial acid degradation. Similar acid degradation and carboxypeptidase rate studies revealed the sequence in peptide LT-3. The sequence of amino acids in peptide C-4 was resolved utilizing subtilisin data and partial acid splitting.

Peptides C-3, C-4, S-2, S-3, S-4, S-7 and S-9 were selected for study of amide linkages since each contained but one such potential group. Only four peptides, C-3, C-4, S-2 and S-9, yielded stoichiometric quantities of ammonia when incubated with concentrated acid. These data are in complete agreement with the determined number of amide groups, and with the behavior of the respective peptides on ion exchange columns.

The sequence in peptide S-8B was determined by a time study with carboxypeptidase.

Integration of these data provides a basis for the complete amino acid sequence of glucagon (*cf.* Table I). The details of this work will be reported in publications now being prepared.

(13) The arrangement of peptides is patterned after that suggested by F. Sanger, *Advances in Protein Chem.*, **7**, 1 (1952).

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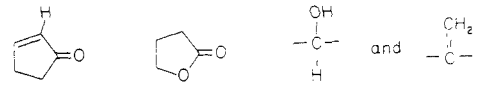
RECEIVED MAY 21, 1956

TERPENES. VI. THE STRUCTURES OF HELENALIN AND ISOHELENALIN

Sir:

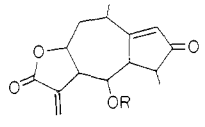
Helenalin ($C_{15}H_{18}O_4$), the active principle of the common sneezeweed, *Helenium microcephalum Linn* was first isolated by Lamson[1] from the related species, *Helenium automnale*. Structurally it did not come under close scrutiny until investigated by R. Adams and W. Herz[2] in 1949. These authors have provided conclusive evidence for the presence of the following structural units in the helenalin molecule



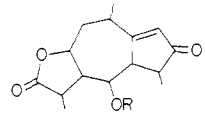
We now wish to discuss a few additional experiments from which we have derived expression I for this natural product. The ultraviolet spectrum of I (λ_{max} 220 mμ, ϵ 12200) is a composite of two isolated chromophores: By subtracting the curve of dihydrohelenalin (III), (λ_{max} 229 mμ, ϵ 6500) from the one of I a curve (λ 210, ϵ 10,000) resulted which is identical with the one due to the unsaturated lactone chromophore present in alantolactone (V).[3] The methylene group in I is thus conjugated with the lactone carbonyl.

It now became necessary to establish the carbon skeleton of I. Tetrahydrohelenalin (VI) was reduced with lithium aluminum hydride to a tetrol which on acetylation was converted to a tetraacetate (m.p. 86–87°, found: C, 62.78; H, 8.16). Catalytic dehydrogenation of this compound over palladium at 305° yielded guaiazulene. Helenalin (I) is therefore a sesquiterpene.

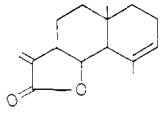
From the following interconversion it became clear that I is a γ-hydroxylactone. A second isomeric substance was present in *Helenium microcephalum* which we would like to name isohelenalin (VIII) (m.p. 260–262°, found: C, 68.69; H, 7.22; λ_{max} 219 mμ, ϵ 20,000, infrared max. in KBr 2.97; 5.76; 5.90; 6.35μ). On catalytic reduction VIII gives dihydroisohelenalin (IX) (m.p. 212°, found: C, 68.23; H, 7.60; λ_{max} 217 mμ, ϵ 14,500, infrared max. in KBr 3.02; 5.79μ) which is resistant to further hydrogenation. The corresponding diketone (X) (m.p. 150–152°, found: C, 68.78; H, 7.05, λ_{max} 234 mμ, ϵ 15,000, infrared max. 5.70; 5.74; 5.98μ) obtained by oxidation of IX with chromic acid on reduction with zinc in acetic acid yields tetrahydrohelenalone (VII).[2]



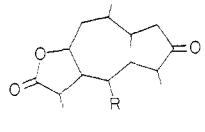
I, R = H
II, R = Ac



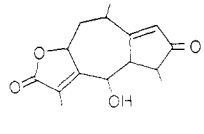
III, R = H
IV, R = Ac



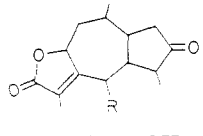
V



VI, R = OH
VII, R = O=



VIII

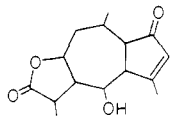


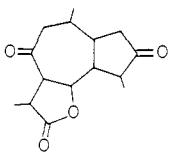
IX, R = OH
X, R = O=

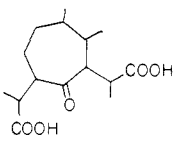
(1) P. D. Lamson, *J. Pharmac. Exper. Ther.*, **4**, 471 (1913).

(2) R. Adams and W. Herz, THIS JOURNAL, **71**, 2546, 2551, 2554 (1949).

(3) L. Ruzicka, P. Pieth, T. Reichstein and L. Ehmann, *Helv. Chim. Acta*, **16**, 268 (1933); W. G. Dauben and P. D. Hance, THIS JOURNAL, **75**, 3352 (1953).


XI


XII



XIII

The cyclopentenone moiety established previously[2] can be placed on the guaiazulene template in two different fashions, leading to III or XI. Quantitative evaluation of the nuclear magnetic resonance spectra of I, II, III and IV to be discussed in detail later, indicate that helenalin is represented by I (*e.g.*, the calculated ratio of C=C—C—H to CH,CH_2 to CH_3 in XI is 4:4:6, in III 2:3:9; found: 2.2:3.2:8.6).

Similarly it was possible to differentiate between the two isomeric ketolactones VII and XII by this method. The calcd. ratio of HC—C=O to CH,-CH_2 to CH_3 in XII is 7:3:9; calcd. for VII is 6:4:9; found: 5.8:3.9:9.5.

Support for the resulting expression VII for tetrahydrohelenalone was found in the base catalyzed cleavage of VII to a dicarboxylic acid, $C_{15}H_{22}O_5$ (λ_{max} 236 mμ, ϵ 7700) first observed by Herz.[4] The ultraviolet spectrum of this compound is very similar to that of Butenandt acid.[5] We would like to propose the following scheme to rationalize this unusual reaction: VII → α,β-unsaturated ketoacid ⇄ trienediol ⇄ conjugated enedione ⇄ vinylogous β-diketone → XIII. The nature of the changes involved in the formation of XIII requires the presence of a 1,5-diketone which is absent in XII.

Acknowledgments.—We are indebted to Eli Lilly and Co. for financial support. Our thanks are due to Prof. J. Waugh for his help with the NMR spectra, to Prof. R. Adams for a sample of helenalin, to Dr. A. Eschenmoser for a gift of alantolactone and to Prof. W. Herz for sending us a copy of his manuscript prior to publication.

(4) W. Herz, private communication.

(5) L. F. Fieser, THIS JOURNAL, **75**, 4386 (1953).

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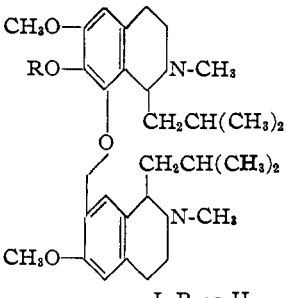
ALKALOID STUDIES. XIV.[1] THE STRUCTURE OF THE CACTUS ALKALOID PILOCEREINE

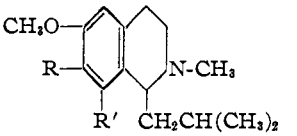
Sir:

In the first paper of this series[2] attention was called to the fact that the alkaloid pilocereine ($C_{30}H_{44}N_2O_4$),[3] isolated from several giant cacti,[2,4] appears to be quite unusual since even the most complicated cactus alkaloid of established constitution (lophophorine)[5] possesses only the empirical formula $C_{13}H_{17}NO_3$. We should now like to report the key experiments which lead to the assignment of structure I for pilocereine.

Cleavage of pilocereine methyl ether (II) with potassium in liquid ammonia[6] led to a mixture of phenolic and non-phenolic fractions readily separable by extraction with alkali. Methylation of the crude phenolic portion (III) with diazomethane in methanol–ether furnished 1-isobutyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (IV) which was identified with a synthetic specimen[7] by infrared comparison of the bases and by mixture melting point determination of the respective picrates. The non-phenolic mixture could be resolved into its components by careful chromatography on acetic acid-deactivated alumina and yielded in order of increasing polarity 1-isobutyl-2-methyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (V), 1-isobutyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (IV) and 1-isobutyl-2-methyl-6,7-dimethoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline (VI). Identity of IV and V with synthetic material[7,8] was established by direct comparison of the respective bases and of their crystalline picrates while VI (kryptophenolic) was first methylated with diazomethane in methanol-ether for nine days to give 1-isobutyl-2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline (VII), which proved to be identical with the base (infrared comparison and undepressed mixture melting point of respective picrates) recently synthesized[8] from mescaline. The isolation of all four possible cleavage products of the methyl ether II confirms unambiguously the skeletal structure of pilocereine. The kryptophenolic character[2] of the alkaloid and the difficulty encountered in methylation require that the free phenolic group be located at C-7 as shown in I. Potassium–ammonia cleavage of pilocereine (I) itself proceeded by a more complicated path since in addition to III[9] and V there were also encountered rearrangement products. These experiments and the results of



I, R = H
II, R = CH_3



III, R = OH; R′ = H
IV, R = CH_3O; R′ = H
V, R = R′ = H
VI, R = CH_3O; R′ = OH
VII, R = R′ = CH_3O
VIII, R = C_2H_5O; R′ = H

(1) Paper XIII, O. O. Orazi, R. A. Corral, J. S. E. Holker and C. Djerassi, *J. Org. Chem.*, **21**, Sept. (1955).

(2) C. Djerassi, N. Frick and L. E. Geller, THIS JOURNAL, **75**, 3632 (1953).

(3) G. Heyl (*Arch. Pharm.*, **239**, 451 (1901)), who first isolated this alkaloid in an amorphous form, assigned to it the $C_{30}H_{44}N_2O_4$ formula while our analytical data of the crystalline base and its derivatives were more compatible (ref. 2) with $C_{30}H_{42}N_2O_4$. The presently described structure elucidation requires the H_{44} formulation.

(4) C. Djerassi, C. R. Smith, S. P. Marfey, R. N. McDonald, A. J. Lemin, S. K. Figdor and H. Estrada, THIS JOURNAL, **76**, 3215 (1954).

(5) For a review on cactus alkaloids see L. Reti, *Progr. Chem. Org. Nat. Prod.*, **6**, 242 (1950).

(6) *Cf.* M. Tomita, *ibid.*, **9**, 175 (1952).

(7) C. Djerassi, J. J. Beereboom, S. P. Marfey and S. K. Figdor, THIS JOURNAL, **77**, 484 (1955).

(8) C. Djerassi, F. X. Markley, R. Ehrlich and R. Mirza, *J. Org. Chem.*, **21**, Sept. (1956). The synthesis of V, VI and VIII was carried out exactly as described in ref. 7 for IV by starting with the appropriate β-phenylethylamine.

(9) Ethylation with diazoethane furnished 1-isobutyl-2-methyl-6-methoxy-7-ethoxy-1,2,3,4-tetrahydroisoquinoline (VIII) which was shown to be identical with a synthetic specimen (ref. 8).