

THE CONSTITUTION OF ECHINULIN

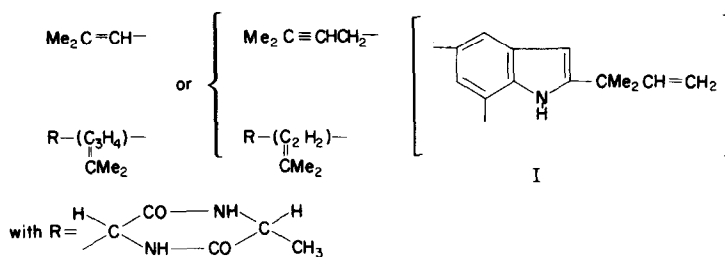
C. Cardani, G. Casnati, F. Piozzi and A. Quilico

Istituto di Chimica Generale del Politecnico, Centro di Chimica

Industriale del C.N.R., Milano

(Received 14 September 1959)

PREVIOUS investigations on echinulin,<sup>1</sup>  $C_{28}H_{37}O_2N_3$ , an important constituent of the mycelium of the moulds of the Aspergillus glaucus group, had led us to assign to this substance partial formula (I).



<sup>1</sup> Previous communications: A. Quilico, F. Piozzi and C. Cardani,

The attribution of a  $-\text{CH}_2-$  group to the alkenic side chain or to the bridge which links the indole and diketopiperazine moieties of the molecule was still uncertain, and the absolute location of the two substituents in 5- and 7-positions of the indole ring remained undetermined.

We succeeded in solving the first of these problems by a thorough re-examination of the oxidative degradation products (fatty acids and non-volatile aliphatic acids) of hydroechinulin,  $\text{C}_{28}\text{H}_{43}\text{O}_2\text{N}_3$ , echinulin itself, and of the 2,5,7-trisubstituted indoles which are formed by alkaline pyrolysis of echinulin and its hydroderivative.<sup>2</sup> The identification of the fatty acids obtained on oxidation in different conditions was achieved by vapour phase chromatography, whereas the non-volatile acids were characterized by paper chromatography and then isolated in amounts sufficient for the chemical identification by chromatography on column of silicagel. Drastic oxidation of hydroechinulin with nitric acid, alkaline permanganate, acidic permanganate, with continual steam distillation of the fatty acids formed, gave the following acids: acetic, isobutyric, isovaleric,  $\alpha,\alpha$ -dimethylbutyric and isocaproic. Of these, the first four acids had been already isolated in earlier degradative work,<sup>3</sup> whereas isocaproic acid had not yet been identified. Its quantity depends on the nature of the oxidant and on the more or less drastic conditions in which the oxidation has been carried out. The obtaining of isocaproic acid indicates

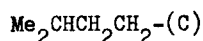
---

Gazz. Chim. Ital. 88, 125 (1958), C. Cardani, G. Casnati, B. Cavalleri and A. Quilico, Rend. Accad. Lincei [8] 24, 488 (1958).

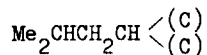
<sup>2</sup> A. Quilico, C. Cardani and F. Piozzi, Gazz. Chim. Ital. 85, 3 (1955).

<sup>3</sup> A. Quilico and C. Cardani, Gazz. Chim. Ital. 83, 155 (1953).

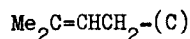
that hydroechinulin contains the C<sub>5</sub> side chain (II) or (III), which must be present in its unsaturated form (IV) or (V) in echinulin itself.



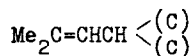
II



III



IV



V

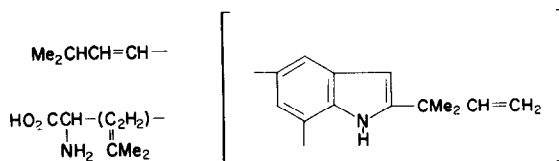
This fact is also confirmed by the isolation, among the non-volatile nitric acid oxidation products of hydroechinulin, of the  $\alpha$ -hydroxy- $\alpha$ -methyl-glutaric acid lactone, m.p. 69°, easily reduced by HI and P to  $\alpha$ -methyl-glutaric acid, m.p. 78°. Considerable amounts of the lactonic acid are also formed on nitric acid oxidation of the indole from alkaline pyrolysis of hydroechinulin, and it can be reasonably assumed that both isocaproic acid and the lactonic acid take their origin from the same side chain (II) or (III).<sup>4</sup>

The demonstration that echinulin actually contains an isopentenyl group (IV) directly attached to the benzene ring has been achieved by the study of the products of its alkaline cleavage. On prolonged treatment at 200° with ethanolic KOH, migration of the double bond of (IV) to the

---

<sup>4</sup> It is known that isocaproic acid yields the  $\alpha$ -hydroxy- $\alpha$ -methyl-glutaric acid lactone by drastic nitric acid oxidation. Cf. J. Bredt, Ber. 14, 1780 (1881).

conjugated position with the benzene system occurs conjointly with the hydrolysis of the diketopiperazine ring, giving alanin and the indole aminoacid isoechinin  $C_{25}H_{34}O_2N_2$  (VI).

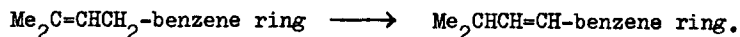


VI

Catalytic hydrogenation of (VI) affords the corresponding saturated hydroechinin,  $C_{25}H_{40}O_2N_2$ , which can also be obtained by hydrolysis of hydroechinulin with ethanolic KOH at  $200^\circ$ . Ozonolysis in acetic acid of iso-echinin yields a mixture of volatile carbonyl compounds; chromatographic analysis on silica gel column of the corresponding 2:4-dinitrophenylhydrazones gave the dinitrophenylhydrazones of formol, acetone and isobutanal in the almost theoretical molecular ratios (1:1:1) required by formula (VI). Echinulin with the same treatment yielded formol and acetone dinitrophenylhydrazones in about 1:2 molecular ratios.

Conclusive evidences for the presence of side chain (IV) and not (V) have been supplied by the study of the U.V. and I.R. spectra of echinulin

and its transformation products. The U.V. spectra of echinulin and its hydroderivative are practically identical, and the same can be said for those of the deoxybases (deoxyechinulin and deoxyhydroechinulin) which are formed on reduction with lithium aluminium hydride.<sup>5</sup> This indicated that none of the three double bonds of the non-hydrogenated products are conjugated with the indole system. On the contrary, strong differences are noticeable in the U.V. spectra of iso-echinin (VI) (in EtOH  $\lambda_{\max}$  255  $\mu$ ,  $\log \epsilon$  4.65;  $\lambda_{\max}$  310  $\mu$ ,  $\log \epsilon$  3.66) and its hydrogenated derivative hydroechinin (in EtOH  $\lambda_{\max}$  228  $\mu$ ,  $\log \epsilon$  4.58;  $\lambda_{\max}$  286  $\mu$ ,  $\log \epsilon$  3.93). The bathochromic shift of the maxima in iso-echinin can be attributed to conjugation of the double bond of the isopentenyl chain with the benzene ring. In hydroechinin the maxima are in the same positions as in echinulin and hydroechinulin. Moreover, the I.R. spectrum of iso-echinin shows a neat band at 965  $\text{cm}^{-1}$  which can be assigned to a trans -CH=CH- group. This band, which is not present in the echinulin I.R. spectrum, demonstrates that the drastic alkaline treatment has caused the shift:



On the basis of similar chemical and spectrochemical evidence we concluded that the unsaturated 2,5,7-trisubstituted indole which is formed in the alkaline pyrolysis of echinulin is actually a mixture of at least two substances of which one contains chain (IV) as in echinulin, and the other is the isobase in which the double bond of (IV) has shifted into conjugation with the

---

<sup>5</sup> A. Quilico, F. Piozzi and C. Cardani, Gazz. Chim. Ital. **88**, 125 (1958).

benzene ring. Indoles with a different degree of unsaturation and possibly with different a number of carbon atoms are probably present in the mixture.

Further information on the structure of the C<sub>5</sub> bridge which unites the indole and diketopiperazine moieties of echinulin is supplied by the fact that both echinulin and its hydroderivative are racemized by sodium ethoxide in ethanol solution at ordinary temperature. This would indicate that echinulin does not contain extra asymmetry centres except the two belonging to the diketopiperazine ring. This permits us to discard structure (VII) which would also give acetone on ozonolysis and leaves structure (VIII) as the only one possible for the bridge.



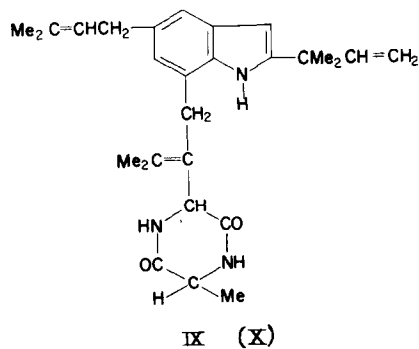
The alkaline pyrolysis of echinulin and hydroechinulin still needs a closer investigation,\* but its results are best accounted for when in (VIII) Y is the diketopiperazine moiety. Taking into account the previous degradative work and other considerations that will be discussed in a more detailed paper, we can safely conclude that echinulin possesses either constitution (IX) or (X), in which the substituents in the 5- and 7-positions of the indole ring have been exchanged.

Other considerations, in particular the obtaining of cryptoechinulinic acid on permanganate oxidation of hydroechinulin,<sup>6</sup> would suggest formula (IX)

---

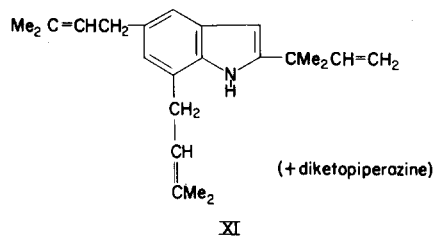
\* Experiments on models are in progress.

<sup>6</sup> A. Quilico and C. Cardani, Gazz. Chim. Ital. **83**, 155 (1953).



as the most probable. The isopentanic nature of the two side chains and of the bridge is evident in both. Echinulin would therefore take its origin from the condensation of an indole containing three isoprene units in 2-, 5- and 7-positions with a molecule of glycine and one of L(+)-alanine.

Note. On the occasion of the meeting of the Tetrahedron Honorary Editorial Advisory Board held in Munich on 4 September 1959, Professor A. J. Birch kindly informed me that biosynthetic work still in progress by himself H. Smith and R. A. Blance with 2-<sup>14</sup>C-mevalonic acid and Me<sup>14</sup>CO<sub>2</sub>H clearly indicates that the most probable skeleton for echinulin is (XI).



This brilliant result is in perfect agreement with the conclusions we have reached on the basis of purely chemical and spectrochemical evidences, and further supports the suggested alternative formulae (IX) and (X). — A.Q.

---