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THE STRUCTURE OF PEDERIN.

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Pederin, m.p. 113°, is the toxic principle isolated in 1952 by Pavan and Bo (1) from Paederus fuscipes Curt.; successively, in the extraction of a larger quantity of insects, we could separate a second product (m.p. 142°) closely related to pederin which we called pseudopederin (2).

Our investigations led us to assign to pederin the molecular formula $C_{25}H_{45}O_9N$ containing 4 $CH_3(C)$, 4 OCH_3, one -NHCO- ; the IR and NMR data summarized below indicate that free OH and cyclic O are also present, and supply further information on the state of the carbon atoms which carry the $CH_3(C)$ groups:

IR (KBr) : OH 3560, 3490 ; CONH 3400, 1665, 1510 ; OH and O-ether series of bands at 1120-1040 (xx)

NMR (acetone-d6) : $>CH-C\underline{H}_3$ 1.18 D, 1.02 D ; $\rightarrow C-C\underline{H}_3$ 0.96 S, 0.87 S ; $O-C\underline{H}_3$ 3.28, 3.30, 3.33, 3.37

Pseudopederin possesses formula $C_{24}H_{43}O_9N$ and contains only 3 OCH_3 consistently with the fact that on gentle heating in aqueous solution pederin is hydrolyzed as shown by the equation (3):

$$C_{25}H_{45}O_9N + H_2O \longrightarrow C_{24}H_{43}O_9N + CH_3OH$$

and in agreement with the IR and NMR data reported below:

IR (KBr) : OH 3500, 3370 (shoulder), 3280 ; CONH 3400, 1648, 1510 ; OH and O-ether series of bands at 1120-1050

(x) Of the S.A. Farmitalia, Research Laboratories, Milano.

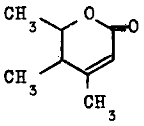
(xx) IR frequencies are expressed in cm^{-1}; chemical shifts in p.p.m. relative to tetramethylsilane as internal standard; J in c.p.s..

NMR (acetone-d6) : $>\mathrm{CH-C}\underline{\mathrm{H}}_3$ 1.03 D, 1.03 D ; $\geq\mathrm{C-C}\underline{\mathrm{H}}_3$ 0.95 S, 0.88 S
$\mathrm{O-C}\underline{\mathrm{H}}_3$ 3.28, 3.32, 3.35

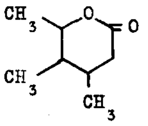
Preliminary degradative work ($KMnO_4$ oxidation, acidic hydrolysis), both on pederin and pseudopederin, gave unsatisfactory results owing the number of the products which were formed and the difficulty of their separation; of significance was the isolation of *oxamic acid* from permanganate oxidation. We wish now to report the results of our further investigations that allowed us to establish the structure of the two products.

Barium methoxide in boiling methanolic solution attacks readily pseudopederin (but not pederin) giving a number of substances of which a neutral and an acidic product are predominant and form together the 80% of the reaction product when the former is carried out in oxygen atmosphere.

The neutral product, pederolactone (m.p. 72-73°), has formula $C_8H_{12}O_2$. It gives a dihydroderivative $C_8H_{14}O_2$ on catalytic hydrogenation, and is split by ozonolysis into oxalic acid and 3-methyl-3-penten-2-one. To pederolactone and hydropederolactone can therefore be assigned structures (I) and (II) which are consistent with the IR, UV and NMR spectra. Both structures have been confirmed by synthesis of the optically inactive lactones.



I



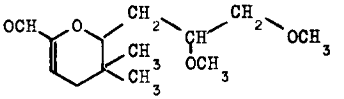
II

The acidic product, *meropederoic acid*, is an oxamic acid with pK_{mcs} 4.18 . With diazomethane it gives a *monomethylester* $C_{17}H_{31}O_8N$ (m.p. 137-138°) which on treatment with acetic anhydride in pyridine yields a *monoacetate* $C_{19}H_{33}O_9N$ (m.p. 102°). The IR spectrum of the acetate (CONH 3311, 1709, 1517 ; CO-acetate 1748, 1248) shows that no free OH is still present.

Cautious hydrolysis of the ester with 0.04 N barium hydroxide restores meropederoic acid. On heating with N/1 HCl the product yields oxalic acid, ammonia, methanol and a liquid aldehyde $C_{13}H_{22}O_4$, *pederenal*, according to the equation:

$$C_{17}H_{31}O_8N + 2\ H_2O \xrightarrow{H^+} (COOH)_2 + NH_3 + 2\ CH_3OH + C_{13}H_{22}O_4$$

which indicates that an extra OCH_3 group besides that of the $COOCH_3$ is split off in the form of methanol during the hydrolysis. Pederenal reacts with two molecules of p-nitrophenylhydrazine giving a bis-p-nitrophenylhydrazone $C_{25}H_{34}O_7N_6$. We could assign to pederenal structure (III) on the base of the following experimental evidences and considerations:

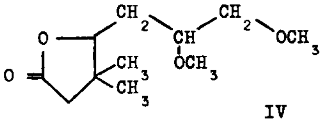


III

a) Its UV spectrum (hexane) has a maximum at 259 mμ (ε 8,300) and its IR spectrum shows bands at 2725 (aldehyde CH), 1695 (CO in α,β-unsat. aldehyde) and 1639 (C=). Moreover its NMR spectrum (CCl_4) contains the following signals: $\succ C-C\underline{H}O$ 9.16 S ; $\succ C-C\underline{H}_3$ 0.91 S, 0.99 S ; $O-C\underline{H}_3$ 3.35, 3.37 ; $>C=CH-CH_2-C\lessdot$ 5.71 (1H, T 4.0) , 2.05 (2H, D 4.0).

b) Ozonolysis of pederenal affords a dicarboxylic acid which on hydrolysis with 2 N HCl gives oxalic acid and a γ-lactone, pedelactone, $C_{11}H_{20}O_4$ (IR : CO for saturated γ-lactone 1783). Permanganate oxidation of this lactone, as well as of pederenal itself, yields α,α-dimethyl-succinic acid.

c) Pedelactone, which is not affected as such by periodic acid, after boiling with aqueous 30% HBr is readely oxidized at room temperature by this reagent yielding formaldehyde. This indicate the presence of a groupment $-CH(OCH_3)-CH_2OCH_3$. Further details on the structure of pedelactone are supplied by the NMR spectrum reported below.



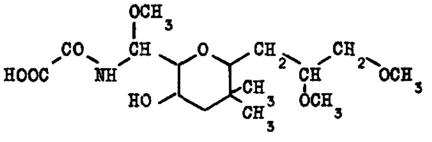
IV

NMR ($CDCl_3$) : $\succ C-C\underline{H}_3$ 1.03 S, 1.17 S
$O-C\underline{H}_3$ 3.35, 3.38 ; $\succ C-C\underline{H}_2-CO$ 2.33 S
$O-\overset{\backslash}{C}H-CH_2-O$ (3H) 3.4-3.7 ; $-CO-O-\overset{\backslash}{C}H-CH_2-$ ABX 4.16 (X) and 1.85 (center of AB).

To pedelactone can therefore be assigned structure (IV), and taking into account its relationships with pederenal, structure (III) can be attributed to this last.

As reported above, the **acidic hydrolysis** of meropederoic acid **yields, besides pederenal, oxalic acid, ammonia** and methanol. This process can be interpreted as the saponification of the amide linkage of an oxamic acid accompanied by the hydrolysis of an aminoacetal groupment and the elimination of an hydroxylgroup located in β-position, which lead to the formation of the -CHO function and the α,β-double bond of pederenal.

On these bases structure (V) can be assigned to meropederoic acid, which is consistent with the results of IR and NMR measurements of its methylester:

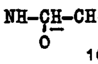


V

Methylester:

IR : OH 3650 ; CONH 3356, 1695, 1515 ; CO-ester 1748

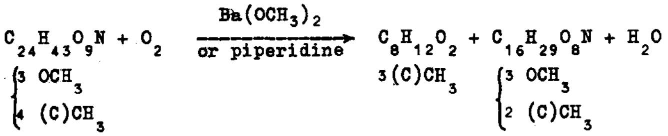
NMR ($CDCl_3$) : NH 7.50 (D 9.5)



$NH{-}\underset{\underset{\textstyle O}{|}}{C}H{-}CH{<}$ 5.30 (DD 9.5, 7.5)

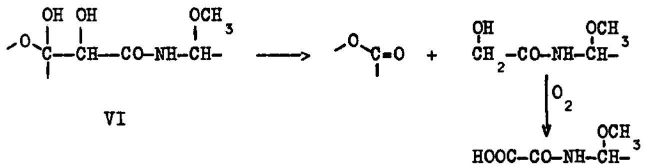
1OH 1.92 S

The cleavage of pseudopederin by barium methoxide with the intervention of atmospheric oxygen can now be expressed by the stoichiometrical equation:

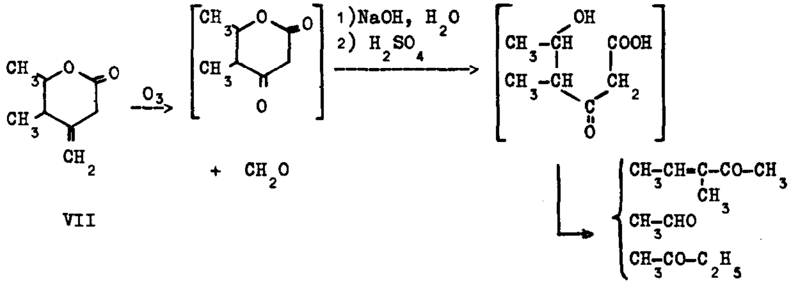


$$\underset{\begin{cases}3\ OCH_3\\4\ (C)CH_3\end{cases}}{C_{24}H_{43}O_9N} + O_2 \xrightarrow[\text{or piperidine}]{Ba(OCH_3)_2} \underset{3(C)CH_3}{C_8H_{12}O_2} + \underset{\begin{cases}3\ OCH_3\\2\ (C)CH_3\end{cases}}{C_{16}H_{29}O_8N} + H_2O$$

Since pederolactone (I) and meropederoic acid (V) are formed from pseudopederin also on treatment with piperidine in oxygen atmosphere, in absence of methanol and barium methoxide, it can be inferred that the three OCH_3 of meropederoic acid were originally present in pseudopederin which, most likely, contained also the two C_5O rings of the cleavage products. The formation of pederolactone and meropederoic acid can easily be accounted for by assuming the intervention of a retroaldolisation process followed by oxidation as shown by schema (VI):



It remains to be explained the appearance of a fifth $(C)CH_3$ group in the balance of the reaction products. The presence of partial structure (VI) is supported by the results of the lead tetraacetate oxidation in benzene solution of pseudopederin. The aqueous extract of the reaction mixture contains a fraction which, on heating with N/1 HCl, yields again pederenal (III). The benzene soluble fraction is mainly formed by a new lactone, isomeric with pederolactone (I). Isopederolactone cannot be purified by chromatography because is quantitatively converted into pederolactone by alumina. To isopederolactone can be assigned structure (VII); in fact on ozonolysis it gives formaldehyde and a ketone (different from the methylpentenone obtained in similar conditions from pederolactone) which, on treatment with sodium hydroxide, affords acetaldehyde, methylethylketone and 3-methyl-3-penten-2-one, as shown below:

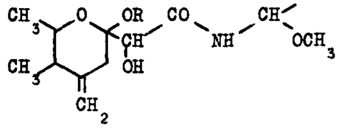


From the formation of isopederolactone (VII) it can safely be concluded that pseudopederin contains a methylene group in position 4 to the oxygen atom of a tetrahydropyrane ring. The extra $(C)CH_3$ of pederolactone (I) takes its origin from the shift of the exocyclic double bond to a position conjugated with the lactonic CO under the influence of barium methoxide or piperidine.

Since pederin resists the action of these last reagents, is not affected by lead tetraacetate in benzene solution, and differs from pseudopederin only for the presence of a OCH_3 easily hydrolyzable by heating in aqueous solution, it can be inferred that partial structure (VIII) is present in pederin; the corresponding moiety of pseudopederin is therefore (IX).

VIII R = CH_3

IX R = H



Accordingly, pseudopederin assumes two hydrogen atoms on hydrogenation in methanol solution with Adams catalyst. Dihydropseudopederin (m.p. 95-96°) on treatment with barium methoxide gives in good yields meropederoic acid (V) and a liquid lactone $C_8H_{14}O_2$ which has proved to be identical with dihydropederolactone (II). The same lactone could be obtained also by lead tetraacetate oxidation of dihydropseudopederin. In the presence of Pd-C catalyst pseudopederin assumes larger amounts of hydrogen yielding, besides dihydropederin, a product $C_{24}H_{45}O_8N$ (m. p. 95°) containing an oxygen atom less than the dihydroderivative. The NMR spectrum (in acetone-d6) of dihydrodeoxypseudopederin shows the following signals for methyl groups: 3 $OC\underline{H}_3$ 3.27, 3.29, 3.31 ; 3 $>CH-C\underline{H}_3$ 0.77 D, 0.97 D, 1.07 D ; 2 $\geq C-C\underline{H}_3$ 0.87 S, 0.95 S .

Similarly, hydrogenation of pederin with Adams platinum affords dihydropederin $C_{25}H_{47}O_9N$ m.p. 120-121° (NMR in benzene-d6 : 4 $O-C\underline{H}_3$ 3.20, 3.25, 3.35, 3.47 ; 3 $>CH-C\underline{H}_3$ 0.77 D, 0.84 D, 1.10 D ; 2 $\geq C-C\underline{H}_3$ 0.90 S, 0.95 S). With Pd-C as the catalyst one molecule of methanol is eliminated by hydrogenolysis and dihydrodeoxypseudopederin is the main reaction product. Both dihydropederin and dihydropseudopederin when heated with N/1 HCl yield pederenal (III) and a neutral product m.p. 85-86° whose formula $C_9H_{18}O_3$ has been confirmed by mass spectrography(xxx). On oxidation with lead tetraacetate it gives formaldehyde and dihydropederolactone (II). To this product, pederol, which reacts easily with

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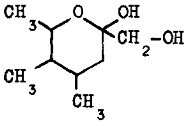
(xxx) By courtesy of Dr. J. Seibl of the E.T.H., Zürich.

p.nitrophenylhydrazine giving an osazone, structure (X) can be assigned:

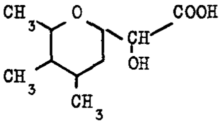


X

IR : CH_3 1375 ; OH 3280, 1140, 1050

cyclic ether 1110

Dihydrodeoxypseudopederin resists lead tetraacetate oxidation and is not affected by barium methoxyde; on hydrolysis with N/1 H_2SO_4 gives pederenal (III) and a carboxylic acid, pedolic acid. Its methylester $C_{11}H_{20}O_4$ (mass spectrography$^{(xxx)}$) does not react with p.nitrophenylhydrazine. Its chemical and spectrochemical behaviour allow to assign to pedolic acid structure (XI).



XI

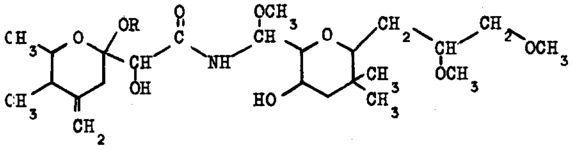
Methylester:

IR : CH_3 1380 ; OH 3450, 1087 ; CO–ester 1750, 1250 ; cyclic ether 1110

NMR (acetone-d6 + D_2O) : 3 $>CH-C\underline{H}_3$ 0.73, 0.93, 1.06 D ; $>CH-C\underline{H}<^{OH}_{CO}$ 4.08 (D 3.0)

While pederin and pseudopederin undergo partial hydrogenolysis to dihydrodeoxypseudopederin in the presence of Pd-C, dihydropederin and dihydropseudopederin do not give dihydrodeoxypseudopederin by similar treatment. This would indicate that once saturated the exocyclic bond of the two substances, the OH (respect. the OCH_3) group adjacent to the O atom of the tetrahydropyrane ring is no more liable to hydrogenolysis. A possible explanation of this fact would consist in the well known capacity of palladium catalysts of promoting migration of double bonds. Thus, if we admit that in the course of the hydrogenation pederin and pseudopederin might partially be transformed into the corresponding isomers containing the double bond in the 3-4 position of the C_5O ring, the OH or OCH_3 on carbon 2 would belong to an allylic system and be therefore easily removed by hydrogenolysis. Actually, when platinum is used as the catalyst, no migration of the double bond practically occurs, and only the stable dihydroderivatives are formed.

On the basis of the experimental evidences and considerations above reported we propose for pederin and pseudopederin respectively the structures (XII) and (XIII):



XII R = CH_3 pederin

XIII R = H pseudopederin

The complete work with the experimental part, including a number of derivatives which have not been reported because were not essential for the demonstration of the structures, as well as the NMR spectra discussion, will be published in Gazzetta Chimica Italiana.

Researches on the stereochemistry of pederin are in progress.

Note. - Recently Matsumoto and co-workers (4), on the basis of our preliminary work and some spectrochemical evidences, have suggested some small fragments of the structure of pederin(*). Only few of them are consistent with the behaviour of this substance and in agreement with the structure proposed by us. Thus, according to Matsumoto and co-workers, pederin should contain 3 OH of which only two are acylable. Diacetylpederin $C_{29}H_{49}O_{11}N$ (m.p. 114°), that we prepared with acetic anhydride in pyridine at room temperature, does not show any OH band in its IR spectrum. The hypothesis that elimination of a third OH could have occurred in this treatment can be discarded since diacetylpederin, on reduction with $LiAlH_4$ in ether, restores pederin.

(*) Matsumoto and co-workers (private communication) had at their disposal only wery small quantities of pederin; for the research here reported we could use about 8.0 grams of this costly product.

Acknowledgements. - We thank prof. M. Pavan who organized the collection of large quantities of the insect and assisted us with biological tests during the extraction of the active substance, and also dr. B. Cavalleri and dr. G. Venturini for their collaboration in some parts of this work.

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