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ON THE METABOLITES OF PENICILLIUM DUCLAUXI DELACROIX -- III*
THE REACTIONS OF DUCLAUXIN WITH AMMONIA AND PRIMARY AMINES. THE STRUCTURES OF DESACETYLDUCLAUXIN, NEOCLAUXIN, XENOCLAUXIN AND CRYPTOCLAUXIN.

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As reported previously(1,2), the structure of duclauxin(I), $C_{29}H_{22}O_{11}$, m.p. 230°(decomp.), $[\alpha]_D^{30}$+ 272.5°(c=5.4, $CHCl_3$), a principal metabolite of Penicillium duclauxi DELACROIX, was established by the X-ray crystallographical analysis of its monobromo derivative. As a characteristic reaction of duclauxin, an orange N-containing pigment is formed by the action of aqueous ammonia. By the action of other primary amines homologous pigmented compounds are also afforded, which have been designated generally "duclauxamides". The two phenolic hydroxyls of duclauxin are not essential for this reaction, since dimethyl ether of duclauxin (II), m.p. 180° (decomp.), yields the same type of N-containing pigment.

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* Part I, ref.(1) and part II, ref.(2).

TABLE I

	Mol.formula	M.p.	$[\alpha]_D^{16}$ [*]
Duclauxamide (V)	$C_{26}H_{17}O_8N$ 1/2H_2O	280°(decomp.)	- 462°
N-Methylduclauxamide (VI)	$C_{27}H_{19}O_8N$ 1/2H_2O	290°(decomp.)	- 507°
N-Ethylduclauxamide (VII)	$C_{28}H_{21}O_8N$ 1/2H_2O	250°(decomp.)	- 493°
N-Methylduclauxamide dimethyl ether (VI')	$C_{29}H_{23}O_8N$ H_2O	300°(decomp.)	

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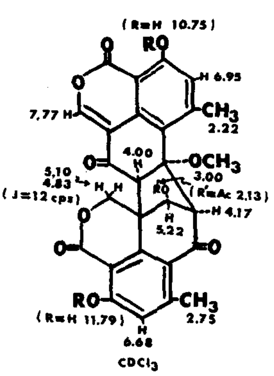
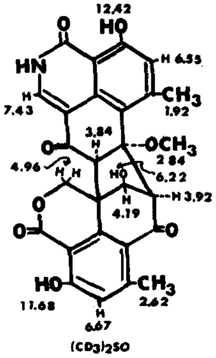
[*] Measured in tetrahydrofuran

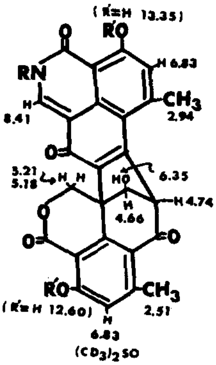
On treatment of duclauxin with aqueous ammonia in a shorter period of time, a colourless intermediate compound, protoduclauxamide, $C_{27}H_{21}O_9N$, m.p. 250°(decomp.), was formed, which was converted into duclauxamide by heating in acetic acid or mineral acid-containing acetone ,or by the action of cold 10% NaOH.

In regard to the molecular formula of protoduclauxamide, it was evident that duclauxamide was formed from the former compound by the loss of CH_3OH . On the other hand, the mild alkaline or acid hydrolysis of duclauxin afforded desacetylduclauxin, $C_{27}H_{20}O_{10}$, m.p. 253°(decomp.). The comparison of n.m.r. spectra of desacetylduclauxin and protoduclauxamide revealed that displacement of O with NH takes place by the action of ammonia without conversion of their original carbon skeleton. Such a displacement has been shown to occur in the isocoumarins (3), and the same system is involved in the molecule of duclauxin.

On the basis of the established structure of duclauxin (I), the structures of desacetylduclauxin (III), protoduclauxamide (IV) and "duclauxamides" (V)~(VII) are formulated as follows:





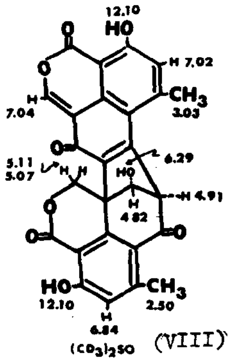


(I) R=H, R'=Ac
(II) R=CH_3,R'=Ac
(III) R=R'=H

(IV)

(V) R=R'=H
(VI) R=CH_3,R'=H
(VI')R=R'=CH_3
(VII)R=C_2H_5,R'=H

On boiling desacetylduclauxin in glacial acetic acid or treating duclauxin with 10% NaOH at room temperature for 5~6 hrs. neoclauxin, $C_{26}H_{16}O_9$, m.p. ca 250°(decomp., softens from 160°), was yielded, whose n.m.r. spectrum is consistent with the formula (VIII).



(VIII)

The structural correlation between neoclauxin (VIII) and duclauxamide (V) was demonstrated by the ready conversion of the former into the latter by the action of aqueous ammonia.

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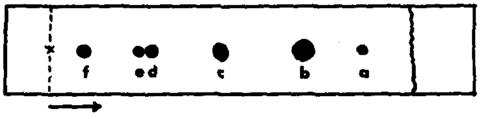
* Unless otherwise mentioned, figures in the structural formulae are the chemical shifts (ppm.) relative to TMS.

The thin layer chromatogram of the acetone extracts of the mycelia of <u>Penicillium duclauxi</u> showed the presence of several metabolites other than the principal product, duclauxin.

FIG. I



Solvent: Benzene:Acetone (9 : 1). Plate: Silica gel G impregnated with oxalic acid

a: Xenoclauxin b: Duclauxin c: Cryptoclauxin
d: Desacetylduclauxin e: Clauxin f: Neoclauxin

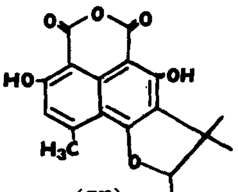
The occurrence of desacetylduclauxin (III) and neoclauxin (VIII) in the fungus was revealed by the spots on the thin layer chromatogram, though they have not actually been isolated as yet, while xenoclauxin, cryptoclauxin and clauxin have been obtained in a pure state.

Xenoclauxin, $C_{28}H_{18}O_{11}$, m.p. $>300°$(decomp.), $[\alpha]_D^{26}$ + 310° (c=1, tetrahydrofuran), a faint yellow compound was isolated by silicic acid column chromatography of the mother liquor separated from duclauxin by the recrystallization.

In contrast with duclauxin , xenoclauxin gave no colouration with aqueous ammonia. This would indicate the absence of isocoumarin system in the molecule of xenoclauxin.

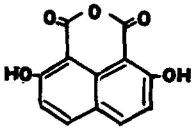
Xenoclauxin showed the u.v. absorption at 241, 288 and 358 mu and a strong blue fluorescence under u.v. light to suggest a perinaphthalic anhydride structure, since the "acid anhydride" (IX) derived from norherqueinone and atrovenetin, the fungal phenalenones of <u>Penicillium herquei</u>

and P. atrovenetum (4a-g), was characterized by these properties. Xenoclauxin gave characteristic i.r. absorption bands at 1723 and 1676 cm^{-1}, while desacetylxenoclauxin trimethyl ether, $C_{29}H_{22}O_{10}$, m.p.>300° , showed the absorption at 1756 and 1713 cm^{-1}. These data accord with those given by o,o'-dihydroxynaphthalic anhydride (X) and its dimethyl ether (XI), respectively.



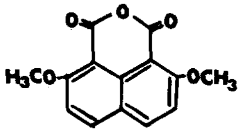
(IX)

I.r.:1703, 1660 cm^{-1}
U.v.256,297,360 mμ



(X)

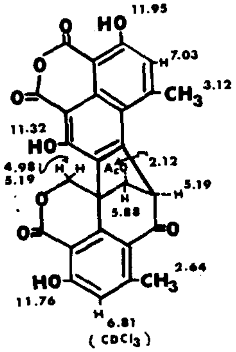
I.r.:1720, 1685 cm^{-1}
U.v.238,330,348 mμ



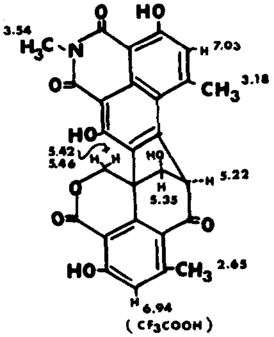
(XI)

I.r.:1750, 1720 cm^{-1}
U.v.245,283,365 mμ

The molecular formula, the u.v.,i.r., and n.m.r. spectroscopical data led to the structure of xenoclauxin as represented by the formula (XII).



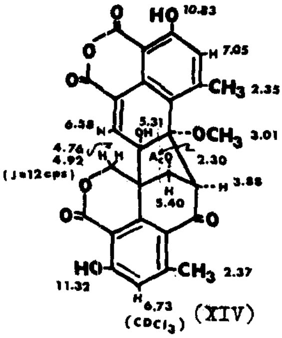
(XII)



(XIII)

The correlation of xenoclauxin and duclauxin was established by the oxidation of the latter into the former with chromic acid in acetic acid-pyridine. On the action of aqueous solution of methylamine, xenoclauxin afforded N-methylxenoclauximide (XIII), $C_{27}H_{19}O_9N$, m.p. 256° (i.r.: 1655, 1620 cm^{-1}), giving an evidence for the acid anhydride structure which,as generally known, reacts with primary amine to give imide. N-Methylxenoclauximide (XIII) was also obtained by the chromic acid oxidation of N-methylduclauxamide (VI). The n.m.r. spectrum of N-methylxenoclauximide showed the signals of 2 aromatic methyls, 1 AB-type methylene, 1 proton attached to the carbon atom bearing secondary hydroxyl, 1 down field shifted methine proton and 2 aromatic ring protons. The signal of N-CH_3 in the naphthalimide portion appeared at δ 3.54, which corresponded to the N-CH_3 signal at δ 3.69 of N-methyl-1,8-naphthalimide.

Cryptoclauxin , $C_{29}H_{22}O_{12}$, colourless needles, m.p. >300° (decomp.), is a minor component of the metabolites of Penicillium duclauxi , which was obtained only in a minute amount. The u.v. absorption (λ_{max} 232.5 mμ(log ε 4.62), 325 mμ(log ε 3.79)) was similar to that of duclauxin, and the i.r. spectrum showed bands at 1800 and 1748 cm^{-1} to indicate the presence of an ordinary acid anhydride system in the molecule. The n.m.r.spectrum of cryptoclauxin which is characterized by the presence of the signals of 1 olefine proton (δ 6.58) and 1 tertiary hydroxyl (δ 5.31,dissapeared



(XIV)

on addition of D_2O) is consistent with the formula (XIV). Experiments on clauxin are now in progress.

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