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NEOECHINULINE: A NEW INDOLE METABOLITE FROM ASPERGILLUS AMSTELODAMI

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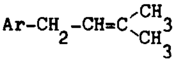
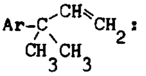
The isolation and study of natural compounds having an isoprene moiety attached to an indole system has acquired momentum due to recent research both on echinuline(1) and especially ergot alkaloids. We have now isolated a new indole derivative neoechinuline (I) from the same moulds which produce echinuline, orange red crystals, mp 237-8° (chloroform), analyzing for $C_{23}H_{25}N_3O_3$(2), obtained from the ether extract of dry micelia of a culture of Aspergillus Amstelodami by absorption chromatography (silica gel, hexane-ethyl acetate 70/30 vol.); uv-vis: λmax. at 230.5 (log ε = 4.51), 287 (4.12) and 420 mμ (3.99); ir (KBr): bands at 3400 (NH); 3200 (NH), 1745 and 1710 cm^{-1} (CO); mass sp.: m/e at 391 (M^+), other important peaks at 376 ($M^+ - CH_3$), 322 ($M^+ - C_5H_9$) and 69 ($C_5H_9^+$). The pnmr spectrum (d_6-DMSO) showed peaks due to acidic protons at 12.07δ(1H), 11.06 (1H) and 9.59 (1H), of which only the last one disappears upon addition of trifluoroacetic acid. A complex feature is present between 6.7 and 7.5 integrating for 4 protons. The presence of two isoprenic units was revealed by the following pnmr data:

(a)  $Ar\text{-}CH_2\text{-}CH{=}C(CH_3)_2$: 3.42δ (2H, d, CH_2); 5.38 (1H, tq, -CH=); 1.75 (6H, d, 2CH_3);
J_{CH-CH_2} = 7.5 Hz; J_{CHCH_3} = 1 Hz.

(b)  $Ar\text{-}C(CH_3)_2\text{-}CH{=}CH_2$: 1.57δ (6H, s, 2$CH_3$); 6.10 (1H, dd, CH); 5.12 and 5.11 (2H, 2dd, =CH_2);
J_{cis} = 11 Hz; J_{trans} = 17 Hz; J_{gem} = 1.5 Hz.

Catalytic hydrogenation (PtO_2, 80 atm., r.t.) of an acetic acid solution of I yielded reduced derivative, hydroneoechinuline, isolated by preparative tlc (silica gel, benzene-ethyl acetate 1/1 vol.), mp 216-8° (ethyl acetate), mol. wt 399 (from mass spectrum: an increase of 8 protons with respect to I), showing a typical indole absorption in the uv spectrum with maxima at 228 (4.57), 285 (3.86) and 295 mμ (3.82) (ethanol). The pnmr spectrum (d_6-acetone) showed peaks at 9.56δ(1H, s, NH), 7.51 (1H, d), 7.11 (1H, d) and 6.83 (1H, dd) respectively for the proton attached to the nitrogen and the aromatic protons of the indolic

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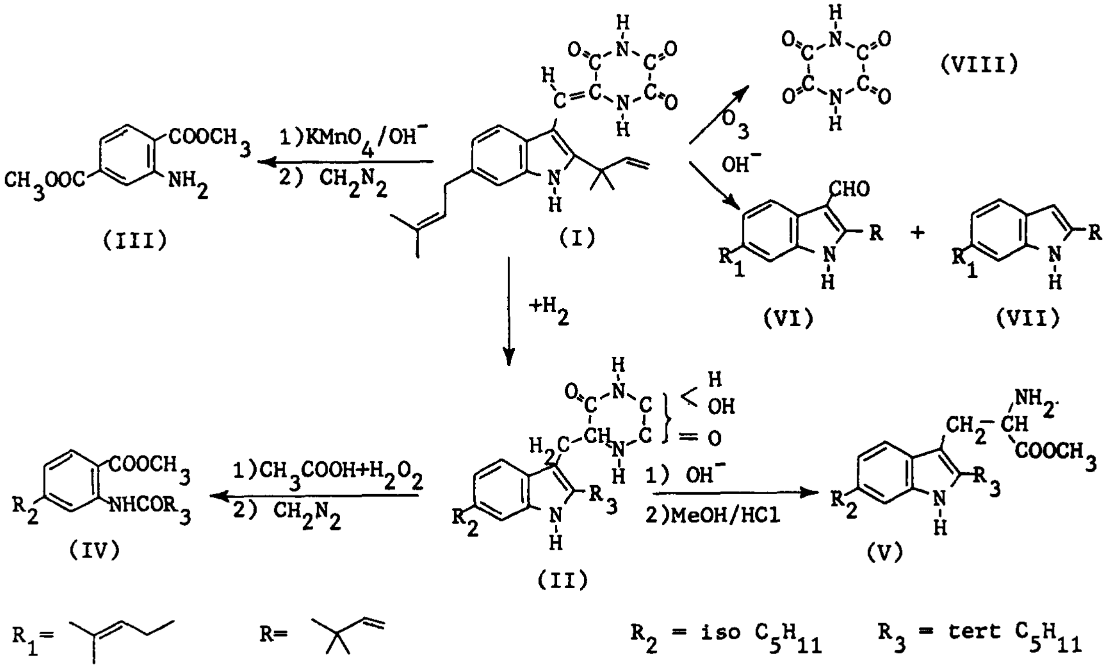
ring. These results and the values observed for the coupling constants are in agreement with a 5- or 6-substitution in the benzene ring.

Chemical cleavage of the compounds afforded defintive evidences as to position of substitution and structure of side chains. Permanganate oxydation of I in basic medium, followed by methylation with diazomethane of the reaction products, gave the methyl ester of 2-aminoterephtalic acid (III), identified by comparison with an authentic sample. Hydrogen peroxide oxydation in cold acetic acid, followed by reduction with zinc dust and methylation yielded oily N-α,α-dimethylbutyrryl-4-isoamylanthranilic acid methyl ester (IV), which had an uv absorption spectrum analogous to that of N-acetyl-4-methylantranilic acid methyl ester and ir bands at 3280 (NH), 1690 and 1550 (amide CO). Its pnmr spectrum (carbon tetrachloride) showed among others, peaks (3H, arom.) at δ 7.85 (d, H_6), 8.66 (d, H_3), 6.79 (dd, H_5) J_{orto} = 8Hz; J_{meta} = 1.5Hz (3); in addition to peaks at δ 11.04 (1H, s, NH) and 3.91 (3H, s, OCH_3), we found a number of peaks indicative of an isoamyl and a t-amyl substituent. Alkaline hydrolysis of IV (40 mg, 1 g of potassium hydroxide, 20 ml water and 30 ml ethanol at 160° during 12 hrs) gave α,α-dimethylbutyrric acid, clearly from the acyl substituent at nitrogen.

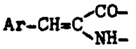
Alkaline hydrolysis of hydroneoechinuline (230 mg, 0.7 g potassium hydroxide in 40 ml ethanol and 1 ml water at 110° during 24 hrs) yielded a product separated by preparative tlc giving a positive ninhydrin test, which was esterified with methanol and hydrogen chloride. The methyl ester obtained had an uv spectrum (ethanol) indicative of an indole structure with maxima at 228 (4.54) and 286 mμ (3.83). The ir bands were located at 3400 (indole NH), 3500 (NH_2) and 1730 cm^{-1} (ester CO), whereas its mass spectrum showed important fragments at m/e 358 (M^+), 326 (M^+-CH_3OH), 299 ($M^+-COOCH_3$), 270 $\left[M^+-CH(NH_2)COOCH_3\right]$, thus effording further evidences forthe identification of the product with 2-t-amyl-6-isoamyltryptophan methyl ester (V). From the alkaline hydrolisis of neoechinuline (400 mg, 1.4 g potassium hydroxide in 55 ml ethanol and 3 ml water at 100-5° during 10 hrs) two main products were isolated by preparative tlc (silica gel, hexane-ethyl acetate 80/20 vol.). The compounds with R_f 0.40 gave a positive test with 2,4-dinitrophenylhydrazine and the following set of physical data : mass spectrum showed important fragments at m/e 281 (M^+), 253 (M^+-CO), 252 (M^+-CHO); pnmr spectrum showed among other, peaks at δ 10.4 (1H, s, CHO) and 8.72 (1H, s, NH); uv (maxima) (4) at λ_{max} 217 mμ (4.51), 249.5 (4.26), 273 (4.05), 312 (4.03), (ethanol).

On the basis of these data, the compound had been identified as 2-t-pentenyl-3-formyl-6-isopentenylindole (VI). The second tlc component, R_f 0.60, gave a strong violet colour with Ehrlich reagent. Its mass spectrum showed a parent peak at 253 (M^+) and other fragments at 238 (M^+-CH_3), 226 (M^+-HCN) and 184 ($M^+-C_5H_9$). The pnmr spectrum, besides the resonances for the aliphatic chains and proton of the indole NH at 7.50δ, showed a singlet for the 3-indole proton (6.1) and signals for aromatic protons: δ 7.25 (d, H_4), 6.90 (d, H_7), 6.72 (dd, H_5); J_{orto}=8 Hz, J_{meta}=1.5 Hz; uv maxima (hexane) at 224.5 mμ (4.47), 270 (3.83), 283

(3.70), 288.5 (3.68), and 295.5 (3.70) supported further evidence for an unconjugated indole structure for the compound. With these data at hand, we can establish structure VII for the compound.



The results of the hydrolysis of hydroneoechinuline, and neoechinuline and the comparison of pnmr spectra of hydroneoechinuline and neoechinuline(5) suggest the partial structure IX for the 3-substituent of neoechinuline. The mass spectrum of (IX) showing a base peak at 270, which can tentatively be identified with indolenin-3-methylene ion(6), a fragmentation common to all tryptophan systems, which is not found in the case of neoechinuline, is also in agreement with (IX)



$$\text{Ar-CH=C}\begin{matrix}\text{-CO-}\\ \text{-NH-}\end{matrix}$$

(IX)

Aldehyde VI formed from IX by a retroaldole condensation, whereas indole VII may arise from alkaline deformylation of VI(7). The definitive structure of the 3-substituent was ascertained by ozonization of neoechinuline with isolation of tetraketopiperazine(8)(VIII).

Structure I is thus definitively established for neoechinuline. Different tautomeric structures are likely for the triketopiperazine moiety. Are left to be determined both the stereochemistry at the dehydrotryptophan bond and the location of saturation upon hydrogenation of the triketopiperazine moiety (compound II).

Neoechinuline is the first(9) natural indole with an isoprenic moiety in 6, the second instance of dehydrotryptophan (10) and the second known natural indole(11) with a t-pentenyl residue attached to the indole system.

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   A. J. Birch and K. R. Farrar, J. Chem. Soc. 4277 (1963).
   E. Houghton and J. E. Saxton, J. Chem. Soc. (C) 1003 (1969).
2. Analytical data for C, H and N for the principal compounds are in agreement with the proposed structures.
3. B. D. Andrews, J. D. Rae and B. E. Reichert, Tetrahedron Letters 1859 (1969) and references therein.
4. Its uv spectrum had the same general features of that of 2-t.amyl-5,7-diisoamyl-3--formylindole.
5. The sequence $-CH_{2(A)}-CH_{(B)}-NH_{(C)}$ was present in hydroneoechinuline [δ 3,54 ($2H_A$, d), 4.17 ($1H_B$, td), 7.96 ($1H_C$, d)(J_{AB}= 7 Hz; J_{BC}= 3.5 Hz)], but not in neoechinuline: shaking with deuterium oxide caused disappearance of the last doublet, while the last but one absorption become a pure triplet.
6. J. H. Benjon, R. A. Saunders and A. E. Williams, in "The mass spectra of organic molecules", p. 303, Elsevier Publ., N.Y. (1968).
7. J. C. Powers, Tetrahedron Letters 655 (1965).
8. An authentic specimen was obtained by ozonization of 2,5-dibenzyliden-3,6-diketopiperazine.
9. Ergot alkaloids contain an isoprene unit in 4, echinuline in 2, 5, and 7.
10. Dehydrotryptophan was first found in the cyclic peptide telomycin: J. C. Sheehan, D. Mania, S. Nakamura, J. A. Stock and K. Maeda, J. Am. Chem. Soc., 90, 462 (1968).
11. The first one is echinuline, see note 1.