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# STRUCTURES OF ISOECHINULINS A, B AND C, NEW INDOLE METABOLITES FROM *ASPERGILLUS RUBER*

Hiromichi Nagasawa*, Akira Isogai, Akinori Suzuki
and Saburo Tamura
Department of Agricultural Chemistry,
The University of Tokyo, Bunkyo-ku, Tokyo, Japan

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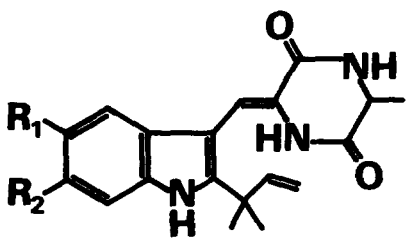
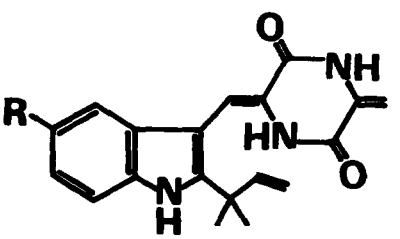
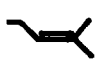
In the course of a continuing search for indole metabolites in mycelia of *Asp. ruber*, three new compounds named isoechinulin A, B and C were isolated and characterized. Based on the chemical and physicochemical evidences, the structures of these compounds have been established as 1, 2 and 3, respectively. Accordingly, the structure 1a preliminarily assigned to isoechinulin A[1] should be revised to 1.





1: $R_1$ =  , $R_2$ = H

2: R = 

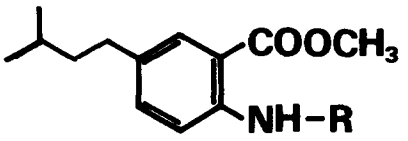
1a: $R_1$ = H, $R_2$ = 

3: R = 

The physicochemical data of isoechinulin A ( IA ) are as follows.
MS m/e : 391.2262 ( $M^+$, $C_{24}H_{29}N_3O_2$ ; calcd. 391.2258 ), 322 ( M-$C_5H_9$ ).
NMR ( in $CD_3COCD_3$ ) δ : 10.21 ( 1H, broad s, -NH- ), 7.92 ( 1H, broad s,

-NH- ), 7.53 ( 1H, broad s, -NH- ), 7.34 ( 1H, d, $J$= 8.2 Hz ), 7.15 ( 1H, s ), 7.10 ( 1H, d, $J$= 1.7 Hz ), 6.99 ( 1H, dd, $J$= 8.2 Hz and 1.7 Hz ), 6.16 ( 1H, dd, $J$= 17.8 Hz and 10.5 Hz, -CH=$CH_2$ ), 5.38 ( 1H, broad t, $J$= 7.1 Hz, -CH=C- ), 5.13 ( 1H, dd, $J$= 10.5 Hz and 1.0 Hz ; 1H, dd, $J$= 17.8 Hz and 1.0 Hz, -CH=$CH_2$ ), 4.28 ( 1H, q, $J$= 6.9Hz, -CH-$CH_3$ ), 3.40 ( 2H, d, $J$= 7.1 Hz, Ar-$CH_2$- ), 1.71 ( 6H, s, -C=C($CH_3$)$_2$ ), 1.55 ( 3H, d, $J$= 6.9 Hz, -CH-$CH_3$ ; 6H, s, -C($CH_3$)$_2$- ). IR $\nu_{max}^{Film}$ $cm^{-1}$ : 3260 ( N-H ), 1675 ( amide C=O ), 1633 ( C=C ). UV $\lambda_{max}^{EtOH}$ nm ( $\varepsilon$ ) : 227 ( 31,500 ), 289 ( 8900 ), 341 ( 9400 ).

Catalytic hydrogenation of IA over $PtO_2$ in acetic acid gave tetrahydro derivative ( 4 ). MS m/e : 395 ( $M^+$ ), 366, 338, 324. In the NMR spectrum of 4, signals at $\delta$ 6.16, 5.38 and 5.13 in IA were unobserved. Appearance of new signals at $\delta$ 0.76 ( 3H, t ) and 0.91 ( 6H, d ) indicated the occurrence of saturation at two isoprene substituents in IA. Oxidative degradation of 4 with hydrogen peroxide in acetic acid at room temperature, followed by methylation with diazomethane, afforded 5 as a colorless oil after purification by preparative TLC. MS m/e : 319 ( $M^+$ ), 262, 248, 221, 216, 164, 71. IR $\nu_{max}^{Film}$ $cm^{-1}$ : 3230 ( N-H ), 1685 ( aromatic ester C=O and amide C=O ), 1520 ( amide N-H ), 1273 ( ester C-O ). NMR ( in $CCl_4$ ) $\delta$ : 10.05 ( 1H, broad s, -NH- ), 8.71 ( 1H, d, $J$= 8.9 Hz ), 7.73 ( 1H, d, $J$= 2.5 Hz ), 7.32 ( 1H, dd, $J$= 8.9 Hz and 2.5 Hz ), 3.90 ( 3H, s, -COOC$H_3$ ), 2.55 ( 2H, t, $J$= 7.6 Hz, Ar-$CH_2$-$CH_2$- ), 1.8-1.3 ( 5H, m ), 1.25 ( 6H, s, -C($CH_3$)$_2$- ), 0.93 ( 6H, d, $J$= 6.4 Hz, -CH($CH_3$)$_2$ ), 0.88 ( 3H, t, $J$= 7.9 Hz, -$CH_2$-$CH_3$ ).




5 : R = CO-

6 : R = H

7 : R = $COCH_3$

Hydrolysis of 5 with hydrochloric acid in methanol under reflux gave 6. MS m/e : 221 ( $M^+$ ), 190, 164, 132. NMR spectrum ( in $CDCl_3$ ) of 6 showed signals due to aromatic protons at $\delta$ 7.65 ( 1H, d, $J$= 2 Hz ), 7.12 ( 1H, dd, $J$= 7.8 Hz and 2 Hz ) and 6.62 ( 1H, d, $J$= 7.8 Hz ). Signals at $\delta$ 2.49 ( 2H, t, $J$= 8.0 Hz ), 1.8-1.2 ( 3H, m ) and

Table 1

| Compound | Chemical shifts of aromatic protons ( coupling constants ) | Solvent |
|---|---|---|
| 5 | 8.71 ( 8.9 ), 7.73 ( 2.5 ), 7.32 ( 8.9, 2.5 ) | $CCl_4$ |
| 6 | 6.62 ( 7.8 ), 7.65 ( 2 ), 7.12 ( 7.8, 2 ) | $CDCl_3$ |
| 7 | 8.60 ( 7.5 ), 7.82 ( 2 ), 7.37 ( 7.5, 2 ) | $CDCl_3$ |
| 8 | 6.43 ( 9 ), 6.97 ( 2 ), 7.54 ( 9, 2 ) | $CCl_4$ |
| 9 | 8.57 ( 8.4 ), 7.71 ( 2 ), 7.26 ( 8.4, 2 ) | $CCl_4$ |

0.92 ( 6H, d, $J$= 5.8 Hz ) suggested the presence of Ar-$CH_2$-$CH_2$-$CH(CH_3)_2$ moiety. Thus, 6 was determined as methyl anthranilate with a 3-methylbutyl group at C-4 or -5 and, consequently, 5 as N-(2,2-dimethylbutyryl) derivative of 6.

N-Acetylation of 6 with acetic anhydride-pyridine gave 7. MS m/e : 263 ( $M^+$ ), 232, 221, 206, 164, 132. This treatment caused a remarkable downfield shift ( from δ 6.62 to δ 8.60 ) in one of the signals due to three aromatic protons in the NMR spectrum of 6, suggesting that this aromatic proton in 6 should be located at C-3 adjacent to the acetylamino group[2]. Accordingly, the location of the 3-methylbutyl group was determined at C-5 in 5, 6 and 7. This conclusion was further supported by the NMR spectra of methyl 5-methyl anthranilate ( 8 ) and its N-acetyl derivative ( 9 ), which were derived from 5-methylindole ( commercially available ) in the same procedure. These data showing the chemical shifts of aromatic protons in 5-9 are listed in Table 1.

The following data were obtained for isoechinulin B ( IB ). MS m/e : 389.2137 ( $M^+$, $C_{24}H_{27}N_3O_2$ : calcd. 389.2102 ), 320 ( M-$C_5H_9$ ). NMR ( in $CD_3COCD_3$ ) δ : 10.23 ( 1H, broad s ), 9.80 ( 1H, broad s ), 8.08 ( 1H, broad s ), 7.30 ( 1H, d, $J$= 8.2 Hz ), 7.2-7.0 ( 2H, broad s ), 6.96 ( 1H, broad d, $J$= 8.2 Hz ), 6.13 ( 1H, dd, $J$= 18.0 Hz and 10.5 Hz ), 5.4-5.0 ( 5H, m ), 3.38 ( 2H, d, $J$= 8.0 Hz ), 1.68 ( 6H, s ), 1.54 ( 6H, s ). IR $\nu_{max}^{Film}$ $cm^{-1}$ : 3310, 1678, 1643. UV $\lambda_{max}^{MeOH}$ nm ( ε ) : 228 ( 27,900 ), 272 ( 18,700 ), 285 sh ( 16,600 ), 370 ( 10,400 ). Catalytic hydrogenation of IB gave a hexahydro

derivative which was identical with the tetrahyrdo derivative of IA ( 4 ) in the IR, MS, UV and NMR spectra. Thus, the structure of IB was determined as 2.

The molecular formula of isoechinulin C ( IC ) was assigned as $C_{24}H_{27}N_3O_3$ by high resolution mass spectrometry ( m/e 405.2108 : calcd. 405.2051 ). MS m/e: 405 ( $M^+$ ), 336 ( $M-C_5H_9$ ), 334 ( $M-C_4H_7O$ ). NMR ( in $CD_3COCD_3$ ) δ : 10.21 ( 1H, broad s ), 9.73 ( 1H, broad s ), 8.12 ( 1H, broad s ), 7.31 ( 1H, d, $J$= 8.2 Hz), 7.20 ( 1H, broad s ), 7.07 ( 1H, s ), 7.02 ( 1H, broad d, $J$= 8.2 Hz ), 6.11 (1H, dd, $J$= 17.7 Hz and 10.6 Hz ), 5.34 ( 1H, s ), 5.08 ( 1H, d, $J$= 17.7 Hz ), 5.05 ( 1H, d, $J$= 10.6 Hz ), 5.00 ( 1H, s ), 3.1-2.7 ( 3H, m ), 1.56 ( 6H, s ), 1.33 ( 3H, s ), 1.22 ( 3H, s ). IR $\nu_{max}^{Film}$ $cm^{-1}$ : 3250, 1676, 1644. UV $\lambda_{max}^{MeOH}$ nm ( ε ) : 229 ( 26,700 ), 272 ( 18,000 ), 286 sh ( 15,800 ), 371 ( 9700 ). Treatment of IB with *m*-chloroperbenzoic acid in chloroform at room temperature afforded a mixture of IC and its stereoisomer at a ratio of ca. 3 : 1 ( observed in the NMR spectrum ). The MS and IR spectra of the mixture showed sufficient identity with those of IC. Accordingly the structure of IC was established as 3.

## References

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