(-)-Mitorubrinic Acid, a New Metabolite of *Penicillium vermiculatum* Dang. F-852

[a]B. PROKSA, [b]T. LIPTAJ, [b]N. PRÓNAYOVÁ, and [a]J. FUSKA

[a]*Department of Biochemical Technology, Faculty of Chemical Technology, Slovak Technical University, SK-812 37 Bratislava*

[b]*Central Laboratories, Faculty of Chemical Technology, Slovak Technical University, SK-812 37 Bratislava*

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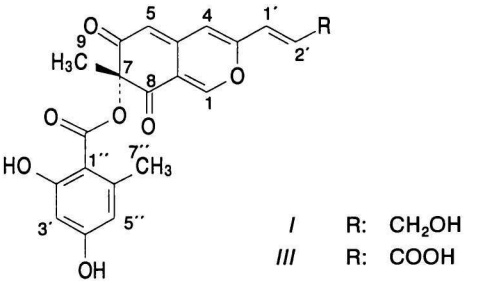
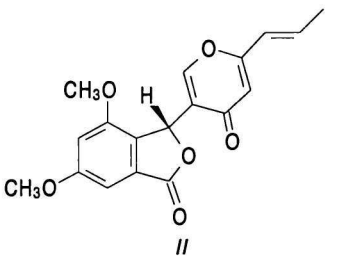
Vermistatin and mitorubrinic acid, together with a degradation product of mitorubrinic acid were isolated from the mutant strain of *Penicillium vermiculatum* cultivated on a saccharose medium. The mechanism of biosynthesis of these compounds is proposed.

Penicillium vermiculatum Dang. F-852 cultivated on the saccharose medium biosynthesized the macrodiolide antibiotic vermiculin and traces of the azaphilone mitorubrinol (*I*) [1]. This strain grown on the glucose medium biosynthesized the phthalidopyranone derivative vermistatin (*II*) only [2]. The new strain, prepared from the parent one by the active selection after UV irradiation biosynthesized, however, compound *II*, but the major metabolite was the new yellow pigment *III* giving the positive azaphilone reaction with ammonia [3]. Compound *III* afforded the similar UV spectrum as mitorubrinol (*I*) did. ^{1}H NMR spectrum of the azaphilone *III* differed from that of compound *I* by absence of the methylene signal at δ = 4.32, manifested also by the signal at δ = 61.9 in the ^{13}C NMR spectrum. On the other hand, the signal of the carboxyl carbonyl appeared in the spectrum of compound *III*; replacement of the hydroxymethyl group by the carboxyl one caused the small differences in the chemical shifts of the side chain carbons (Table 1). Because the shifts of the other carbons in the spectrum of *III* were in good accord with those in the mitorubrinol spectrum we were entitled to ascribe the structure of mitorubrinic acid to azaphilone *III*. A new method for the determination of *II* and *III* in the cultivation broth was elaborated. According to the results of this analysis, *II* appeared in the medium after 72 h, *III* — mitorubrinic acid not before the 96th h of cultivation. However, biosynthesis of *III* proceeds more rapidly and after 164 h of cultivation concentration of *II* and *III* in medium was 0.63 g dm^{-3} and 1.08 g dm^{-3}, respectively. Extension of cultivation up to 400 h increased the amount of both compounds by another 20 %.

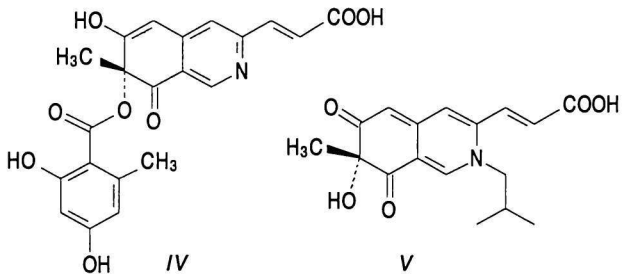
Table 1. ^{13}C NMR Data (δ) of the Studied Compounds

C	*I*	*III*
1	155.3	155.0
3	156.9	152.8
4	110.4	111.1
4a	145.0	142.0
5	107.6	108.7
6	194.0	192.0
7	85.7	85.2
8	193.5	191.6
8a	115.5	115.7
9	22.7	21.8
1′	120.1	133.6
2′	139.8	124.6
3′	61.9	166.1
1″	104.3	104.7
2″	165.8	162.7
3″	101.3	100.6
4″	163.8	162.3
5″	112.5	114.1
6″	144.9	142.4
7″	24.2	22.6
8″	170.6	168.1





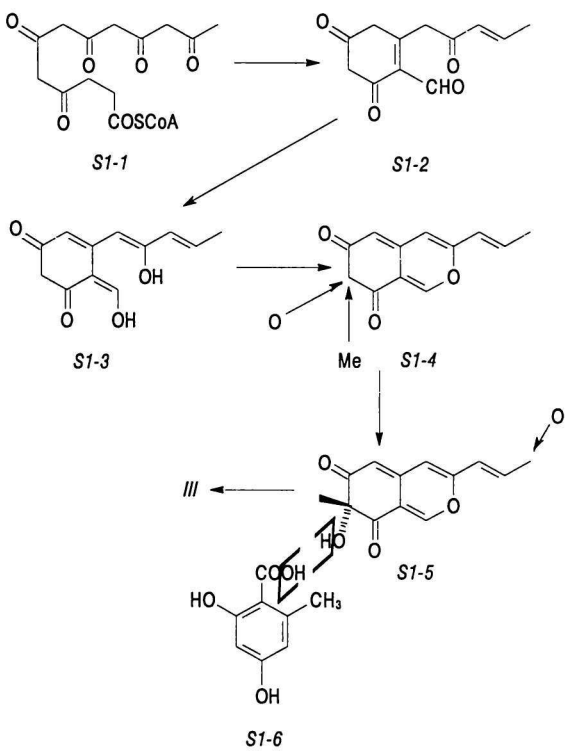
Reaction of mitorubrinic acid (*III*) with ammonia gave the red compound *IV*. The ester grouping connecting the orsellinic acid moiety with that of 6*H*-benzo[2]pyran one was not attacked in this reaction as proved by the NMR spectra. The replacement of oxygen with a NH grouping in the heterocycle caused substantial changes in the UV spectrum of compound *IV* compared with that of mitorubrinic acid (*III*). Due to possible enlargement of conjugation the bathochromic shift of the last absorption band from λ = 301 nm (*III*) to 485 nm (*IV*) was observed. The significant shift of signal of H-5 proton was found in ^{1}H NMR spectrum; its value (δ = 5.37) indicates the presence of the hydroxyl group attached to carbon C-6. This was confirmed in the ^{13}C NMR spectrum, where only one ketone signal was observed. According to all these data acid *IV* exists in the enol form.



While vermistatin (*II*) remained unchanged during the prolonged cultivation, mitorubrinic acid (*III*) was partially transformed into compound *V*. NMR spectrum of this compound lacked the signals of orsellinic acid moiety; presence of the signals of 2-methylpropyl grouping attached to a nitrogen in this spectrum indicates, that acid *III* after hydrolytic cleavage of the ester grouping reacted with 1-amino-2-methylpropane generated probably from valine by decarboxylation.

It is apparent that compounds *II* and *III* are biogenetically related. This hypothesis confirmed also the occurrence of both metabolites in the same cultivation. The most of the known azaphilones are biosynthesized through the polyketide mechanism [4—7]. Mitorubrinic acid (*III*) is composed of the carbinol *S1-5* (Scheme 1) esterified by the orsellinic acid (*S1-6*). Intermediate *S1-5* is formally derived from the hexaketide *S1-1*, which is condensed into ketoaldehyde *S1-2*. Enol form of this aldehyde (*S1-3*) cyclized into substituted 7,8-dihydro-6,8-dioxo-6*H*-benzo[2]pyran (*S1-4*) is then oxidized and methylated at C-7 giving thus carbinol *S1-5*, which esterified with orsellinic acid affords mitorubrin. Oxidation of its side chain generates mitorubrinol (*I*) and mitorubrinic acid (*III*).

Biosynthesis of vermistatin proceeds similarly. Octaketide *S2-1* (Scheme 2) is condensed into aldehyde *S2-2*; its enol forms *S2-3*/*S2-4* dehydrate into



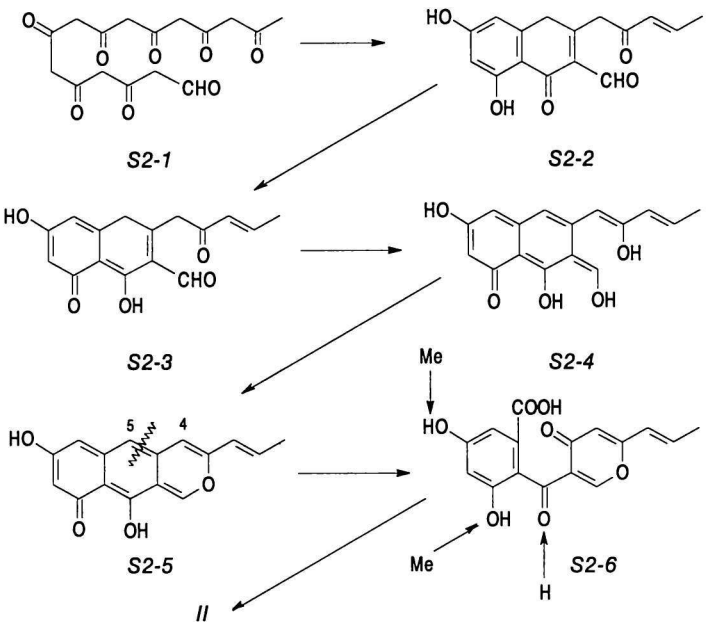
Scheme 1. Biosynthesis of mitorubrinic acid (*III*)

condensed pyran *S2-5*; oxidative cleavage of C-4a—C-5 bond generates benzoyl-pyranone derivative *S2-6*, which is reduced, methylated and lactonized into vermistatin. It is supposed that the analogue of pyran *S2-5*, nectriachrysone, isolated from *Nectria haematococca* [8] is a precursor of rapicone, a benzoylpyranone structurally related to intermediate *S2-6* [9].

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage, the UV spectra were measured with Specord 40 M (Zeiss, Jena), ^{1}H and ^{13}C NMR spectra were recorded with a Varian model VXR-300 spectrometer at 300 MHz and 75 MHz, respectively. HPLC equipment (Laboratory Instruments, Prague) comprised an HPP 5001 pump, LCI 30 injector, LCD 2040 UV detector and CI-105 integrator; column: 150 mm × 3 mm, packed with Separon SGX C18 7 μm (Tessek, Prague); mobile phase: methanol—water (φ_r = 60 : 40, pH 3 adjusted with H_3PO_4); flow rate: 0.4 cm^3 min^{-1}; wavelength of the UV detector: 270 nm. For TLC plates Silufol UV-254 (Kavalier, Votice, CR) were used in the system chloroform—methanol (φ_r = 9 : 1) visualized at 254 nm, with ammonia or spraying with vanillin/sulfuric acid.

Strain *Penicillium vermiculatum* Dang. F-852 was used for cultivation. Composition of the substrate and cultivating conditions were the same as in the production of vermiculin [10].



Scheme 2. Biosynthesis of vermistatin (*II*)

Isolation of Metabolites

Cultivation broth (1.5 dm^3) was centrifuged, supernatant (1.1 dm^3) was acidified to pH 2.0 and extracted with ethyl acetate (three times, 250 cm^3 each), organic layers were combined, dried and concentrated. The residue crystallized from methanol afforded crude vermistatin (*II*, 450 mg). Methanol from the crystallization liquor was evaporated and the residue chromatographed on silica gel column eluted with chloroform—methanol mixture (φ_r = 19 : 1) afforded compounds *III* (R_f = 0.36, 930 mg) and *V* (R_f = 0.50, 63 mg). Both compounds were purified by preparative TLC on silica gel.

Mitorubrinic acid (*III*): m.p. 223—226 °C, [α](D, 20 °C, c = 0.025 %, ethanol) = – 440°; for $C_{21}H_{16}O_9$ (M_r = 412.4) w_i(calc.): 61.17 % C, 3.91 % H; w_i(found): 61.09 % C, 3.96 % H. UV spectrum (CH_3OH), λ_{max}/nm (ε/(dm^2mol^{-1})) 270 (2270), 301 (1420), 346 (1598); λ_{min}/nm: 240 (1280), 294 (1393), 312 (1311); UV spectrum in 0.01 M-KOH (CH_3OH): λ_{max}/nm (ε/(dm^2 mol^{-1})): 243 (1653), 311 (2076), 352 (2718), 506 (417); λ_{min}/nm: 231 (1570), 268 (860), 323 (2050), 410 (109). ^{1}H NMR spectrum, δ: 8.30 (s, 1H, H-1), 7.26 (d, 1H, H-1′, $J_{1',2'}$ = 15.8 Hz), 7.12 (s, 1H, H-4), 6.42 (d, 1H, H-2′), 6.26 (d, 1H, H-5′′, $J_{3'',5''}$ = 2.6 Hz), 6.20 (d, 1H, H-3′′), 5.72 (s, 1H, H-5), 2.64 (s, 3H, H-7′′), 1.56 (s, 3H, H-9). ^{13}C NMR spectra are given in Table 1.

Compound *V*: for $C_{17}H_{19}NO_5$ (M_r = 317.3) w_i(calc.): 64.34 % C, 6.04 % H, 4.41 % N; w_i(found): 64.26 % C, 6.10 % H, 4.43 % N. UV spectrum (CH_3OH), λ_{max}/nm (ε/(dm^2 mol^{-1})): 277 (609), 459 (1140); λ_{min}/nm: 260 (559), 330 (252). UV spectrum in 0.01 M-KOH (CH_3OH): λ_{max}/nm (ε/(dm^2 mol^{-1})): 264 (818), 400 (1574), 563 (676); λ_{min}/nm: 244 (738), 345 (430), 453 (148). ^{1}H NMR spectrum, δ: 8.14 (s, 1H, H-1), 7.05 (s, 1H, H-4), 6.81 (s, 1H, H-5), 6.46 (d, 1H, H-1′, $J_{1',2'}$ = 13.8 Hz), 6.30 (d, 1H, H-2′), 2.80 (m, 2H, N—CH_2), 1.7 (m, 1H, CH), 0.92 (d, 6H, 2 × CH_3), 1.64 (s, 3H, H-9).

Reaction of Mitorubrinic Acid with Ammonia

Mitorubrinic acid (*III*, 300 mg) in methanol (5 cm^3) was mixed with ammonium hydroxide (0.5 cm^3, 25 %) 1 h. Solvents were evaporated *in vacuo*, the residue dissolved in water was acidified with acetic acid to pH 4.0 and red aqueous solution was extracted with ethyl acetate. Organic layer was dried, solvent was evaporated and residue chromatographed on silica gel plates afforded compound *IV* (87 mg); R_f = 0.32; for $C_{21}H_{17}NO_8$ (M_r = 411.4) w_i(calc.): 61.31 % C, 4.17 % H, 3.40 % N; w_i(found): 61.29 % C, 4.22 % H, 3.38 % N. UV spectrum (CH_3OH), λ_{max}/nm (ε/dm^2 mol^{-1})): 279 (2715), 368 (1275), 485 (251); λ_{min}/nm: 240 (1010), 329 (579), 422 (188). UV spectrum in 0.01 M-KOH (CH_3OH): λ_{max}/nm (ε/dm^2 mol^{-1})): 304 (4404), 336 sh (1790), 490 (265); λ_{min}/nm: 256 (1060), 397 (112). UV spectrum in 0.05 M-HCl (CH_3OH), λ_{max}/nm (ε/dm^2 mol^{-1})): 290 (2897), 379 (593); λ_{min}/nm: 241 (1076), 354 (530). ^{1}H NMR spectrum, δ: 8.29 (s, 1H, H-1), 7.36 (d, 1H, H-1′, $J_{1',2'}$ = 16.0 Hz), 7.16 (s, 1H, H-4), 6.75 (d, 1H, H-2′), 6.23 (d, 1H, H-5′′, $J_{3'',5''}$ = 2.5 Hz), 6.13 (d, 1H, H-3′′), 5.37 (s, 1H, H-5), 2.50 (s, 3H, H-7′′), 1.51 (s, 3H, H-9).

Determination of Metabolites *II* and *III* in Cultivation Medium

Cultivation medium (3.00 cm^3) acidified with HCl

(c = 0.5 mol dm^{-3}, 0.2 cm^3) was thoroughly mixed with ethyl acetate (2.0 cm^3, 5 min), suspension was centrifuged (10 000 min^{-1}, 3 min), and 3 mm^3 of the supernatant were injected onto the chromatographic column. A linear relationship between peak area and concentration of the determined compounds in the range of 20—300 μg cm^{-3} was observed with the regression coefficient r better than 0.985 for both compounds. Results were calculated for n = 5, α = 0.05.

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Azolylquinazolines, Synthesis and Biological Activity

[a]M. BODAJLA, [a]Š. STANKOVSKÝ, [a]K. ŠPIRKOVÁ, [b]S. JANTOVÁ, and [b]D. HUDECOVÁ

[a]*Department of Organic Chemistry, Faculty of Chemical Technology, Slovak Technical University, SK-812 37 Bratislava*

[b]*Department of Microbiology, Biochemistry, and Biology, Faculty of Chemical Technology, Slovak Technical University, SK-812 37 Bratislava*

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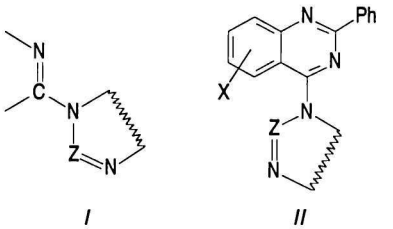
Preparation of some 2-phenyl-4-(azol-1-yl)quinazolines by reaction of corresponding chloroquinazolines with sodium salt of azoles is described. The IR, UV, and ^{1}H NMR spectra and the preliminary screening of biological activity of final products are presented.

The systems in which the 1,2,4-triazole skeleton is connected to the imidoyl grouping (*I*) showed a number of interesting biological effects. The phytoeffectorial one is the most significant of them [1, 2].

In order to extend the series of these compounds, the present communication describes syntheses of some 4-(azol-1-yl)quinazolines (*II*) in which imidoyl grouping is latent being the part of pyrimidine skeleton. At the same time, for the reason of biological activity chlorine was introduced at the benzene ring (Formulae 1).

The syntheses of final compounds started from the chosen 2-phenyl-4-chloro-, 2-phenyl-4,6-dichloro-, and 2-phenyl-4,6,8-trichloroquinazolines, respectively, obtained by the classic methods using anthranilic acid as starting material [3—7].



I *II*

Z = N, CH

X = H, 6-Cl, 6,8-diCl

Formulae 1