Isolation and Structure of a New 1-Decalone Derivative, Rapiculine, from *Ramichloridium apiculatum*[1]

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A new compound, designated rapiculine **1**, was isolated along with fumigatin oxide **2** and spinulosin **3** from the culture filtrate of *Ramichloridium apiculatum* incubated in Czapek–Dox medium supplemented with 0.2% yeast extract, whereas only fumigatin oxide **2** was isolated from the culture filtrate of the same fungus cultivated in potato-dextrose medium. The structure of rapiculine **1** was elucidated by both spectroscopic means and its chemical reactions.

Ramichloridium apiculatum (Miller *et al.*) de Hoog, which is one of the terrestrial hyphomycetes, has been recorded from forest soil in Georgia, USA, from other soils in Pakistan, India and Taiwan, from various plant materials in South Africa, Sri Lanka, India and Australia, and from human skin in Atlanta, USA. The species occurs frequently as a culture contaminant in warmer climates.[2] *R. apiculatum*, strain NHL 2956, was isolated as airborne spores from the packaging room of a bakery in Nagoya, Japan, by H. Tsubouchi in September 1985. This is the first record of the species from Japan.

This fungus was grown in stationary culture on potato-dextrose medium for 3 weeks at 28 °C. The methylene dichloride (CH_2Cl_2) extract of the culture filtrate acidified to pH 2 showed strong activity against *Bacillus subtilis*. The antibacterial metabolite effective against *B. subtilis* was identified as fumigatin oxide **2**, having weak activity against *Escherichia coli*, originally isolated from *Aspergillus fumigatus* Fresenius.[3] When the fungus was cultivated in Czapek–Dox medium supplemented with 0.2% yeast extract for 3 weeks at 28 °C, a metabolite (compound **1**), which was a new type of natural product, was isolated from the CH_2Cl_2 extract of the culture filtrate acidified to pH 2, along with traces of fumigatin oxide **2** and spinulosin **3**, a known metabolite derived from fumigatin oxide **2**, and originally isolated from *Penicillium spinulosum* Thom.[4] The structural elucidation of compound **1**, which we have designated rapiculine, is reported in this paper.

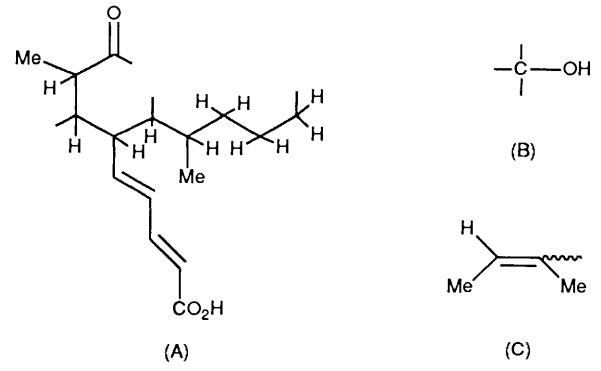
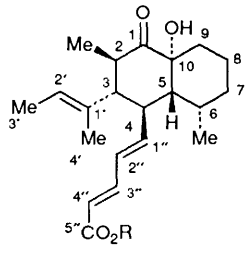


Fig. 1 Partial structures of rapiculine **1**



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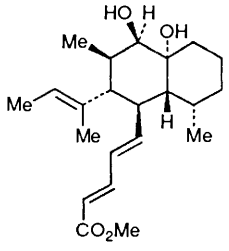

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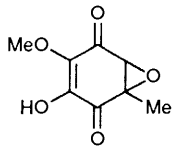

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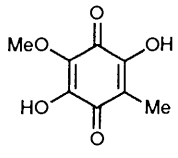

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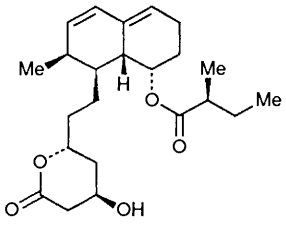

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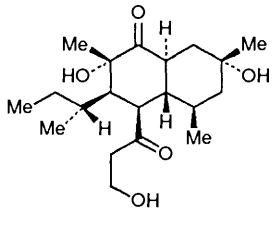

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Results and Discussion

The molecular formula of rapiculine **1** was established as $C_{21}H_{30}O_4$ by high-resolution mass spectrometry and elemental analysis. The IR absorption regions at 3300–2500, 1700 and 1680sh cm^{-1} and the ^{13}C NMR signals at δ_C 171.80 and 217.18 indicated the presence of a carboxylic acid and an aliphatic ketone in compound **1**. The ^{13}C NMR spectrum revealed the presence of 8 sp^2 carbons, participating in two carbonyl groups and thus three carbon–carbon double bonds. Hence the molecule is bicyclic.

The structural fragment (A) shown in Fig. 1 was established by the above results, and the ^1H–^1H correlated (^1H–^1H COSY) and ^1H–^{13}C correlated (^1H–^{13}C COSY) 2D NMR experiments of compound **1**. From the above result and the analysis of the ^1H and ^{13}C NMR spectra, the remaining atoms in rapiculine **1** were assigned to the other two fragments (B) and (C) shown in Fig. 1. Two olefinic double bonds conjugated with the carboxylic acid were both *E* as indicated by the size of the 1″-H/2″-H and 3″-H/4″-H coupling constants (14.9 and 15.4 Hz, respectively). After methylation of compound **1** with diazomethane (CH_2N_2), the ketone moiety of the methyl ester **4** was reduced with $NaBH_4$ in MeOH containing a small amount of

Table 1 ^1H and ^{13}C NMR chemical-shift assignments of rapiculine **1** in $CDCl_3$

Carbon	δ_C	δ_H	J(H,H)/Hz
1	217.18 (Sm)		
2	41.48 (Dm)	2.832 (dq)	10.5, 6.4
2-Me	13.65 (Qt)	1.007 (d)	6.4
3	53.42 (Dm)	2.437 (dd)	10.5, 9.8
4	40.71 (Dm)	2.879 (dd)	11.5, 10.7, 9.8
5	45.01 (Dm)	2.098 (dd)	11.5, 4.6
6	29.72 (Dm)	1.868 (m)	
6-Me	14.13 (Qm)	0.995 (d)	7.1
7	33.35 (Tm)	1.40–1.65 (m)	
		1.621 (br d)	13.7
8	16.26 (Tm)	1.40–1.65 (m)	
		1.918 (ddd)	13.7, 3.5, 3.5
9	35.11 (Tm)	1.331 (ddd)	13.7, 13.4, 4.1
		1.765 (br d)	13.7
10	76.05 (Sm)		
1′	134.11 (Sm)		
2′	118.81 (Dbr s)	5.227 (br q)	6.9
3′	13.28 (Qd)	1.566 (br d)	6.9
4′	16.30 (Qm)	1.548 (br s)	
1″	145.93 (Dbr s)[a]	5.954 (dd)	14.9, 10.7
2″	123.82 (Dm)[b]	6.188 (dd)	14.9, 11.0
3″	146.66 (Ddd)[a]	7.306 (dd)	15.4, 11.0
4″	129.66 (Dbr t)[b]	5.804 (d)	15.4
5″	171.80 (Sbr d)		

[a,b] Assignments may be reversed.

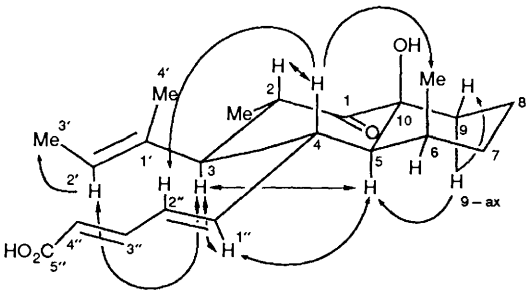

Fig. 2 Difference NOEs of rapiculine **1**

benzene to afford compound **5**. A signal from the proton attached to a carbon bearing a secondary hydroxy group in compound **5** appeared as a doublet (J 5.1 Hz) at δ_H 3.053. In the ^1H–^1H COSY spectrum of compound **5**, the signal at δ_H 3.053 was only connected with that of 2-H that existed in the aliphatic region (δ_H 1.84–1.92). Considering the above result and the chemical shift (δ_C 217.18) of the ketone carbon in the ^{13}C NMR spectrum of compound **1**, a neighbour atom of the ketone should be the carbon in fragment (**B**) (Fig. 1).

A signal for the 1-methyl protons of fragment (C) (Fig. 1) in compound **1** at δ_H 1.513 ([$^2H_6$]acetone) was a triplet due to allylic coupling (J 1.0 Hz). When the signal at δ_H 2.656 in compound **1** was irradiated the signal of the above methyl group changed into a doublet. Thus the structure of rapiculine was assigned as **1**. The ^1H NMR and ^{13}C NMR assignments are provided in Table 1.

The relative stereochemistry was determined by difference nuclear Overhauser effect (DNOE) experiments (Fig. 2). Irradiation of the signal for the 2′-H (δ_H 5.227) brought about enhancement of the 3-H signal (δ_H 2.437) and the methyl signal at C-3′ (δ_H 1.566). When the 3-H signal (δ_H 2.437) was irradiated, enhancement of the 2′-H signal (δ_H 5.227) was observed, along with enhancements of the 1″-H (δ_H 5.954) and 5-H (δ_H 2.098) signals. The 1-methylprop-1-enyl group attached at C-3 must, therefore, have the *E*-configuration for the double bond. The homonuclear ^1H–^1H NOE correlation (^1H–^1H NOESY) spectrum of compound **1** in [$^2H_6$]acetone showed cross-peaks among the 3-H (δ_H 2.656), 5-H (δ_H 1.946) and 1″-H (δ_H 6.069) signals and between the 2-H (δ_H 2.745) and 4-H (δ_H 2.936) signals. The above results and the coupling constants of the 2-H, 3-H, 4-H and 5-H signals (Table 1) indicated that a cyclohexanone moiety in compound **1** existed in the chair conformation. One of the hydrogens at C-9 (δ_H 1.331) was irradiated, causing signal enhancement for 5-H (δ_H 2.098) along with strong enhancement of the other C-9 hydrogen (δ_H 1.765). The coupling constants of the proton at δ_H 1.331 clearly showed that this is an axial proton, related in a 1,3-diaxial manner to the hydrogen at C-5 (δ_H 2.098). We therefore concluded that rapiculine **1** was a *trans*-decalone derivative.

The only problem remaining to be solved was the configuration of the C-6 methyl group. Irradiation of the signal for the 4-H (δ_H 2.936) of compound **1** in [$^2H_6$]acetone caused enhancement of the signals of the above methyl group (δ_H 1.050) and 2″-H (δ_H 6.234). Considering that the 4-H proton was axial and that the coupling constant between the 5-H and 6-H was 4.6 Hz, the C-6 methyl group must be in an axial position. The structure of rapiculine was thus assigned as depicted in structure **1** with the relative stereochemistry shown.

Recently, decaline-based compounds, such as betaenones[5,6] and compactin **6**,[7] having a variety of biological activities, were isolated from micro-organisms. However, rapiculine **1** was found by us not to show antibacterial activity against *B. subtilis* or *E. coli*. These compounds were proposed to be biosynthesized through a polyketide pathway.[8,9] It was assumed that rapiculine **1** was formed from a nonaketide, indicating a different cyclisation manner from that leading to compactin; rapiculine therefore has one more acetate unit than the betaenones [*e.g.*, betaenone B 7[5]].

Experimental

M.p.s were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotation was measured with a JASCO DIP-181 spectrometer. Mass spectra were taken with a JEOL JMS-D-300 spectrometer. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^1H (399.78 MHz) and ^{13}C (100.43 MHz) NMR spectra were recorded on a JEOL JNM-GX-400 spectrometer, and/or ^1H NMR (270.17 MHz) spectra were taken with a JEOL JNM-GX-270 spectrometer, with tetramethylsilane as internal standard. Coupling patterns are indicated as follows: singlet = s, doublet = D or d, triplet = T or t, quartet = Q or q, multiplet = m and broad = br. Capital letters refer to the pattern resulting from directly bonded coupling ($^1H_{C,H}$). J-Values are in Hz. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low-pressure liquid chromatography (LPLC) was performed with a Chemco Low-Prep 81-M-2 pump and glass column (150 or 200 × 10 mm) packed with silica gel CQ-3 (30–50 μm; Wako). TLC was conducted on precoated Kieselgel 60 F_{254} (Art. 5715; Merck). Spots on TLC were detected by their absorption under UV light.

Isolation of Fumigatin Oxide **2** *from* Ramichloridium apiculatum.—*R. apiculatum*, strain NHL 2956, was cultivated at 28 °C for 3 weeks in potato-dextrose medium (6.5 dm^3; 250 cm^3 in each Roux flask). The dried mycelium (51 g) was extracted with chloroform to give ergosterol after purification with column chromatography [benzene–acetone (50 : 1, v/v)]. The culture filtrate (6.5 dm^3) was extracted with methylene dichloride at pH 2 (4 mol dm^{-3} HCl). The evaporated residue (610 mg) was chromatographed on silica gel with chloroform–ethanol (40 : 1, v/v) followed by purification by LPLC [hexane–ethyl acetate–acetic acid (50 : 5 : 1, v/v)] to obtain fumigatin oxide **2** (140 mg) as needles, m.p. 72–74 °C.[3]

Isolation of Rapiculine **1** *from* Ramichloridium apiculatum.—*R. apiculatum*, strain NHL 2956, was grown on still culture at 28 °C for 3 weeks using Czapek–Dox medium supplemented with 0.2% yeast extract (40 dm^3; 250 cm^3 in each Roux flask). The culture filtrate was acidified to pH 2 (4 mol dm^{-3} HCl) and extracted with methylene dichloride. The extract was evaporated to give a residue (2.4 g), which deposited crystals of spinulosin **3** (650 mg) from $CHCl_3$. Compound **3** was recrystallised from benzene, to give purple-black microcrystals, m.p. 198 °C.[4,10] The mother liquid was subjected to silica gel chromatography and elution with benzene–acetone (20:1, v/v), followed by LPLC with the same solvent system to give rapiculine **1** (24 mg), and then fumigatin oxide **2** (2 mg).

Rapiculine **1** was obtained as needles (from benzene), m.p. 199 °C; $[\alpha]_D^{23}$ −73° (*c* 0.35, MeOH) (Found: C, 72.9; H, 8.8%; M^+, 346.2150. $C_{21}H_{30}O_4$ requires C, 72.80; H, 8.73%; M, 346.2144); *m/z* (EI) M^+ (3%), 328.2047 $[(M - H_2O)^+$, $C_{21}H_{28}O_3$ requires *m/z*, 328.2039, 8], 318.2179 $[(M - CO)^+$, $C_{20}H_{30}O_3$ requires *m/z*, 318.2194, 7], 204 (40), 110 (61), 97 (100) and 55 (71); *m/z* 347 [CI (isobutane)] $[(M + 1)^+$, 37%] and 329 $[(M - H_2O + 1)^+$, 100]; λ_{max}(MeOH)/nm 263 (log ε 4.52); ν_{max}(KBr)/cm^{-1} 3370 (OH), 3150–2700 (CO_2H) and 1700 and 1685sh (CO); $\delta_H[(CD_3)_2CO]$ 0.985 (3 H, d, *J* 6.6, 2-Me), 1.050 (3 H, d, *J* 7.3, 6-Me), 1.273 [1 H, ddd, *J* 14.4, 14.4, and 4.4, 9-H (ax)], 1.35–1.60 (4 H, m, 7- and 8-H_2), 1.500 (3 H, br d, *J* 6.8, 3′-H_3)], 1.513 (3 H, t, *J* 1.0, 4′-H_3), 1.77–1.92 [2 H, m, 6- and 9-H (eq)], 1.946 (1 H, dd, *J* 11.1 and 4.5, 5-H), 2.656 (1 H, dd, *J* 10.1 and 8.1, 3-H), 2.745 (1 H, dq, *J* 10.1 and 6.6, 2-H), 2.936 (1 H, ddd, *J* 11.1, 10.5 and 8.1, 4-H), 5.177 (1 H, br q, *J* 6.8, 2′-H), 5.790 (1 H, d, *J* 15.4, 4″-H), 6.069 (1 H, dd, *J* 15.1 and 10.5, 1″-H), 6.234 (1 H, dd, *J* 15.1 and 11.0, 2″-H) and 7.199 (1 H, dd, *J* 15.4 and 11.0, 3″-H). The ^1H and ^{13}C NMR data in $CDCl_3$ are shown in Table 1.

Methylation of Rapiculine **1** *with Diazomethane.*—Rapiculine **1** (15 mg) was methylated with CH_2N_2 in diethyl ether at room temperature. The evaporated reaction mixture was purified by LPLC to give rapiculine methyl ester **4** (13 mg) as an amorphous powder, $\delta_H(CDCl_3)$ 0.984 (3 H, d, *J* 7.1, 6-Me), 1.000 (3 H, d, *J* 6.4, 2-Me), 1.283 (1 H, m), 1.539 (3 H, br s, 4′-H_3), 1.576 (3 H, br d, *J* 6.5, 3′-H_3), 1.26–2.20 (7 H, m), 2.395 (1 H, dd, *J* 10.5 and 10.5, 3-H), 2.64–2.86 (2 H, m, 2- and 4-H), 3.740 (3 H, s, CO_2Me), 5.218 (1 H, br q, *J* 6.5, 2′-H), 5.796 (1 H, d, *J* 15.4, 4″-H), 5.867 (1 H, dd, *J* 15.0 and 10.5, 1″-H), 6.170 (1 H, dd, *J* 15.0 and 9.9, 2″-H) and 7.236 (1 H, dd, *J* 15.4 and 9.9, 3″-H).

Reducation of Rapiculine Methyl Ester **4** *with Sodium Borohydride.*—Rapiculine methyl ester **4** (10 mg) was dissolved in benzene (1 cm^3), and MeOH (4 cm^3) and then sodium borohydride (20 mg) were added. The solution was kept for 10 min at 0 °C before being evaporated to give a yellow oil. After ice–water and 1 mol dm^{-3} HCl (0.2 cm^3) had been added to the residue the solution was extracted with CH_2Cl_2. The extract was evaporated and the residue was chromatographed by LPLC [benzene–ethyl acetate (15:1, v/v)] to yield *dihydrorapiculine methyl ester* **5** (6 mg) as an amorphous powder (Found: M^+, 362.2459. $C_{22}H_{34}O_4$ requires M, 362.2457); *m/z* (EI) M^+ (3%), 344 $[(M - H_2O)^+$, 33], 326 $[(M - 2H_2O)^+$, 26], 245 $[(M - 117)^+$, 98], 227 $[(M - H_2O - 117)^+$, 100] and 55 (98); λ_{max}(MeOH)/nm 266 (log ε 4.45); ν_{max}(KBr)/cm^{-1} 3450 (OH), 1710 and 1700 (CO), 1630, 1260 and 1000; $\delta_H(CDCl_3)$ 1.036 (3 H, d, *J* 7.3, 6-Me), 1.089 (3 H, d, *J* 6.8, 2-Me), 1.21–1.73 (5 H, m), 1.494 (3 H, br s, 4′-H_3), 1.553 (3 H, br d, *J* 6.6, 3′-H_3), 1.769 (1 H, dd, *J* 10.5 and 4.8, 5-H), 1.84–1.93 (3 H, m, 2- and 6-H), 2.177 (1 H, s, OH), 2.412 (1 H, br dd, *J* 8.1 and 7.8, 3-H), 2.633 (1 H, s, OH), 2.709 (1 H, ddd, *J* 10.5, 10.1 and 8.1, 4-H), 3.053 (1 H, d, *J* 5.1, 1-H), 3.773 (3 H, s, CO_2Me), 5.391 (1 H, br q, *J* 6.6, 2′-H), 5.767 (1 H, d, *J* 15.4, 4″-H), 6.078 (1 H, dd, *J* 15.0 and 10.3, 2″-H), 6.152 (1 H, dd, *J* 15.0 and 10.1, 1″-H) and 7.234 (1 H, dd, *J* 15.4 and 10.3, 3″-H).

Acknowledgements

We are grateful to Dr. H. Tsubouchi of Nagoya City Health Research Institute for the supply of the fungal isolate. We are also grateful to Mrs. T. Ogata, Mrs. M. Yuyama and Miss T. Takahashi of Hoshi University for elemental analyses and mass and NMR measurements.

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Paper 0/04090H
Received 7th September 1990
Accepted 23rd October 1990