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Cyclocitrinol, a new fungal metabolite from *Penicillium citrinum*

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Penicillium strains are known for the production of numerous terpenes and nitrogen-containing diketopiperazine-type compounds [1–4]. During our continuing work on metabolites of *Penicillium citrinum* and *P. piscarium* we disclosed a new metabolite which was given the name cyclocitrinol (**1**). Its unusual sesterpenoid structure is composed of two linked bicyclic ring systems. Here we report isolation, structure elucidation and antimicrobial activity of **1**.

The fungal strains of *Penicillium citrinum* VKM F-253, F-3013 and F-3053 were obtained from the All-Russian Culture Collection VKM (Pushchino near Moscow, Russia). Cultivation occurred under submerged conditions (24 °C, rotary shaker, 220–240 rpm) in 750 ml Erlenmeyer flasks containing 150 ml of a medium composed of (g/l): mannitol (50), succinic acid (5.4), $MgSO_4 \cdot 7\,H_2O$ (0.3) and KH_2PO_4 (1) [4]. The culture broth was extracted twice by $CHCl_3$ (1 : 1) and **1** was isolated from the residue of the evaporated extract by a series of chromatographic steps involving column chromatography on normal and reverse phase silica gel. The new metabolite (10 mg) was thus isolated from 5 l of culture broth as a waxy solid. The IR spectrum suggested the presence of double bonds (1613 cm^{-1}) and a keto group (1650 cm^{-1}). Observed optical rotation ($[\alpha]_D^{25} = +169.1°$) and Cotton effects at 245 and 310 nm suggested the chiral nature of **1**.

The molecular weight and the chemical formula of **1** were readily determined by HREI-MS (M^+: m/z 400.2638; calcd. 400.2618 for $C_{25}H_{36}O_4$) suggesting the presence of eight double bonds and/or ring structures. This conclusion was confirmed by ESI-MS displaying m/z 423.0 ($[M + Na]^+$), m/z 823.4 ($[2M + Na]^+$) and m/z 401.4 ($[M + H]^+$). In the EI-MS diagnostic fragments such as m/z 382.2508 (M^+-H_2O; $C_{25}H_{34}O_3$, calcd. 382.2508) and m/z 286.1927 (M^+-side chain cleaved between C-16 and C-19; $C_{19}H_{26}O_2$; calcd. 286.1932) were visible. The structure (relative stereochemistry) of **1** was assigned on the basis one- and two-dimensional ^1H and ^{13}C NMR measurements (COSY, DEPT, HSQC, HMBC, NOESY, for the data see Experimental part). Thereby $^1H,^1H$ COSY and C,H long-range coupled spectra (HMBC) were of pivotal importance. The 2D NMR experiments thus settled unambiguously the sequence of proton and carbon atoms (Fig.)

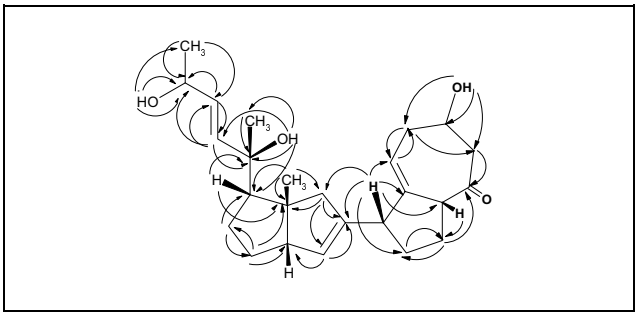
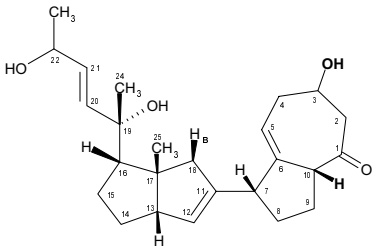


Fig.: Instructive C,H-long-range correlations in the HMBC-spectrum of **1**



and excluded the presence of an ophioboline-type molecule [5].

The ^1H NMR spectrum displayed four olefinic protons coupled either with another olefinic proton (H-20/H-21) or with aliphatic protons (H-5/H-4, H-12/H-13). The value of $^3J_{H\text{-}20,\,H\text{-}21} = 16.5$ Hz suggested the **E**-configuration of the double bond. Moreover, in the ^1H NMR spectrum of **1** three CH_3 groups (0.7 ppm, 1.08 ppm and 1.21 ppm) were visible appearing as doublets and long-range-coupled singlets, respectively. For structural assignment *via* the ^1H, ^1H-COSY and HMBC spectra the hydroxyl proton signals at 4.05 ppm (22-OH), 4.18 ppm (19-OH) and 4.58 ppm (3-OH) were particularly helpful.

The ^{13}C NMR spectrum displayed the presence of one keto group (204.29 ppm), three double bonds and three heteroatom-coupled carbons (63.31, 66.50 and 73.46 ppm). The unusual deep-field shift of C-16 (60.30 ppm) cannot be explained by an ether linkage between C-16 and C-19 due to the hydroxyl proton signal at 4.18 ppm (19-OH) which showed instructive C,H long-range couplings with C-19, C-20, C-24 and C-16 in the HMBC spectrum. Moreover, the linkage of the two bicyclic ring systems via C-7 and C-11 was confirmed by the C,H long-range coupling pattern in the HMBC spectrum of **1** (Fig. 2) and the absence of $^3J_{H-5,\,H\text{-}7}$ and $^3J_{H\text{-}7,\,H\text{-}18}$ couplings in the COSY spectrum. Otherwise couplings of H-4 with H-5, and of H-7 with H-8 were visible.

The location of the keto and hydroxyl groups in a seven-membered ring was attested by the chemical shift data of C-1, C-2, C-3 C-4 and C-10 and by the C,H long-range correlations in the HMBC spectrum, too.

The relative stereochemistry of **1** was proposed by the observable NOE correlations between H-13 and H-25, H-16 and H-24, H_B-18 and H-7, H-7 and H-10, respectively.

The physico-chemical data thus suggest cyclocitrinol (**1**) as a new fungal sesterterpene metabolite possessing a new carbon skeleton. The substance displayed moderate antibacterial activity against some Gram-positive bacteria such as *Bacillus subtilis* ATCC 6633 in the agar plate diffusion assay in concentrations >50 μg/per agar well.

Experimental

1. Instruments

HREI-MS was carried out on an AMD 402 sector field mass spectrometer and ESI-CID-MS/MS on a Quattro instrument (VG Biotech, Altrincham, England). NMR spectra were recorded on a Bruker Avance DRX 500 NMR spectrometer. Optical rotation as measured on a Propol Polarimeter (Dr. Kernchen Optics, Seelze, Hannover) and CD spectrum was recorded on a Jasco instrument.

2. Cyclocitrinol (1)

Yield: 10 mg from 5 l culture broth; waxy material, TLC: R_f 0.55 ($CHCl_2$-MeOH; 9 : 1, v/v, silica gel, Merck, blueish staining by 1% vanillin in conc. H_2SO_4. MS (70 eV) m/z (rel. int.): 400.2638 (M^+, 60), 286.2 (100), 115 (60), 97 (35). IR (KBr, cm^{-1}): 3405, 2940, 1650, 1613, 1455, 1362, 1336, 1178, 1145, 1106, 1067, 1026, 976, 863. ^1H-NMR (DMSO-d_6, δ, ppm): 0.71 (d, $^4J = 1.0$ Hz, 3 H, H-25), 1.08 (dd, $^3J = 6.3$ Hz, $^4J = 1.5$ Hz, 3 H, H-23), 1.21 (s, 3 H, H-24), 1.39 (m,br, 1 H, H_A-15), 1.41 (m, 1 H, H_B-

15), 1.45 (dd, $^2J = 15$ Hz, $^3J = 5.1$ Hz, 1 H, H_A-2), 1.48 (m, 1 H, H_A-8), 1.59 (m,br, 1 H, H_B-14), 1.60 (m, br, 1 H, H_B-14), 1.62 (dd, $^3J = 7.0$ Hz, 2.5 Hz, 1 H, H-16), 1.78 (m, br, 1 H, H_B-8), 2.05 (dd, $^2J = 17.0$ Hz, $^3J = 3.5$ Hz, 1 H, H_A-4), 2.06 (dd, $^2J = 15$ Hz, $^3J = 2.0$ Hz, H_B-2), 2.08 (m, br, 1 H, H-13), 2.17 (d, $^2J = 17.0$ Hz, 1 H, H_A-18), 2.18 (d, $^2J = 17.0$ Hz, 1 H, H_B-18), 2.34 (dd, $^2J = 17$ Hz, $^3J = 5.5$ Hz, 1 H, H_B-4), 2.43 (m, br, 1 H, H_A-3), 2.48 (m, br, 1 H, H_B-3), 2.64 (dd, $^3J = 1.5$ Hz, 2.0 Hz, 1 H, H-10), 2.78 (dd, $^3J = 12.0$ Hz, 6.1 Hz, 1 H, H-9), 3.1 (m, br, 1 H, H-3), 4.05 (q,dd, $^3J = 6.5$ Hz, 5.5 Hz, 4 Hz, 1 H, H-22), 4.18 (s, 1 H, 19-OH), 4.52 (d, $^3J = 6.5$ Hz, 1 H, 22-OH), 4.58 (d, $^3J = 2.8$ Hz, 1 H, 3-OH), 5.38 (d, $^3J = 0.5$ Hz, 1 H, H-12), 5.51 (dd, $^3J = 6.1$ Hz, 1.0 Hz, 1 H, H-5), 5.53 (ddd, $^3J = 16.5$ Hz, 5.5 Hz, $^4J = 1.8$ Hz, 1 H, H-21), 5.64 (dd, $^3J = 16.5$ Hz, $^4J = 2.5$ Hz, 1 H, H-20), ^{13}C-NMR (DMSO-d_6, δ, ppm): 14.52 (C-25), 22.3 (C-14), 22.5 (C-15), 24.20 (C-23), 27.36 (C-7), 27.70 (C-8), 29.10 (C-24), 36.17 (C-4), 39.0 (C-18), 41.56 (C-2), 46.11 (C-13), 48.30 (C-6), 53.45 (C-9), 55.5 (C-13), 60.12 (C-16), 63.31 (C-3), 66.50 (C-22), 73.46 (C-19), 122.2 (C-10), 124.73 (C-12), 131.21 (C-21), 136.20 (C-20), 145.90 (C-5), 157.30 (C-11), 204.29 (C-1). $[\alpha]_D^{25}$ (MeOH, 2.993 mg/ml): +169.1°. CD (Δε, nm): +9.2 (245), −51.1 (310).

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Alkaloids and bioactivity of *Papaver triniifolium*

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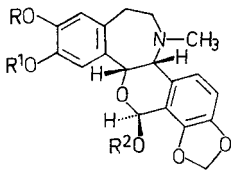
Our previous studies on the endemic species of the section Miltantha, *Papaver triniifolium* Boiss. (Papaveraceae) growing in Turkey revealed the existence of several chemotypes which yielded rhoeadine, morphinane, aporphine and proaporphine types as major alkaloids [1]. Recently the existence of a chemotype containing medicinally important (−)-α-narcotine and papaverine as major alkaloids has been shown [2]. In this work, we report the isolation and characterization of the alkaloids of another sample of *Papaver triniifolium* collected from Beypazarı in Ankara.

Table: Brine shrimp bioassay results of the tertiary and quaternary alkaloidal extracts and compounds 1, 2, 7, 8 of the aerial parts of *P. triniifolium*

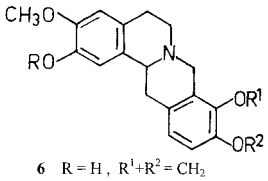
Type of Extracts	ppm	LC_{50}
Tertiary alkaloidal extract	1000 : 100 : 10	515.02
Quaternary alkaloidal extract	1000 : 100 : 10	518.24
1	250 : 25 : 2.5	297.04
2	250 : 25 : 2.5	236.91
7	250 : 25 : 2.5	>1000
8	250 : 25 : 2.5	375.28
Berberine chloride*	250 : 25 : 2.5	8.63

* positive control

The major alkaloids of the aerial parts of this sample have been shown to be rhoeadine [(+)-oreogenine (**1**), (+)-rhoeagenine (**2**)] type. Other alkaloids are three rhoeadines [(+)-oreodine (**3**), (+)-O-ethyloreogenine (**4**), (+)-O-ethyl-rhoeagenine (**5**)], four protoberberines [(−)-cheilanthifoline (**6**), (−)-sinactine (**7**), (−)-*N*-methylsinactine (**8**), (−)-isocorypalmine (**9**)], one phthalideisoquinoline [(−)-α-narcotine (**10**)] and one benzylisoquinoline [crykonisine (**11**)]. This is the first report of the isolation of **1**, **2**, **8**, **9**, **11** from *P. triniifolium* and **8**, **9**, **11** from the section Miltantha. The presence of **8** and **11** has been shown in the Papaveraceae family for the first time. Previously, compound **8** has been isolated from *Fumaria officinalis* (Fumariaceae), whereas compound **11** has been obtained from



1 R = R^1 = CH$_3$, R^2 = H
2 R+R^1 = CH$_2$, R^2 = H
3 R = R^1 = R^2 = CH$_3$
4 R = R^1 = CH$_3$, R^2 = C$_2$H$_5$
5 R+R^1 = CH$_2$, R^2 = C$_2$H$_5$



6 R = H, R^1+R^2 = CH$_2$
7 R = CH$_3$, R^1+R^2 = CH$_2$
9 R = H, R^1 = R^2 = CH$_3$