Isolation and Structures of Minor Metabolites, Cotylenins H and I

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Minor fungal metabolites, cotylenins H and I, were isolated from the culture filtrate of an unidentified species of Cladosporium and their structures have been assigned as II and I, respectively, on the basis of the chemical and spectroscopic evidence.

In a previous paper we reported the isolation and identification of cotylenins F and G, which are diterpene glycosides produced by an unidentified species of Cladosporium. The former is one of the major metabolites which include cotylenins A, C and E. Recently we have isolated two minor metabolites, cotylenins H and I, from the culture filtrate, and found that cotylenins and their aglycone (cotylenol) markedly stimulate the germination of lettuce seeds at a very low concentration of 0.2 ppm in the presence of 5 ppm abscisic acid. This paper reports on the isolation and structural elucidation of the new cotylenins, H and I.

The ethyl acetate extracts of the culture filtrate were chromatographed on silica gel with an acetone–benzene mixture (1:4). Fractions between cotylenins A and C gave three major spots detectable on a TLC plate (Kieselgel, acetone–hexane (3:5)) when sprayed with a solution of vanillin-sulfuric acid. One of these spots agreed with that of cotylenin B on TLC plates. The new cotylenins were carefully separated by silica gel column chromatography with acetone–hexane (1:3). One (less polar than cotylenin B) was further purified by preparative TLC and was crystallized from methanol-water to give colorless needles (cotylenin I, mp 91−4°C, [α]D +3°C (MeOH)). The other (more polar than cotylenin B) was purified by preparative TLC to give a colorless solid (cotylenin H, [α]D +29°C (MeOH)).

Cotylenin I had a molecular formula of C33H54O9, which was determined by elemental analysis and high resolution mass spectrometry. The mass spectrum showed intense peaks at m/e 397 (C22H37O6), 287, 151 and 135, indicating the presence of the sugar residue at C9 in the aglycone cotylenol (III). Expectedly, several characteristic signals observed in the NMR spectrum of cotylenin I (Table I) were very similar to those of the other cotylenins. The additional signals of an ABX system at a lower field and the singlet of six protons at δ 1.31 suggested the presence of a 1,1-dimethylallyl substituent; this was supported by the existence of ions m/e 69 (C5H9O) and 507 (C28H43O8 (M+−18−69)) in the mass spectrum.

Oxidation of cotylenin I with potassium periodate, followed by treatment with 1 N sodium hydroxide in ethanol-water, yielded the
aglycone, mp 156°C, which was identified as III by mixed mp and IR spectrometry. Treatment of cotylenin I with dilute hydrochloric acid at 36°C gave a hydrolysis product which was identified as cotylenin (E) by comparison of TLC, IR and optical rotation. These facts suggested that the isopentenyl group is linked to a hydroxyl group (C2 or C4) in 6-O-methyl-β-D-glucopyranosyl moiety. Cotylenin I was partially acetylated with acetic anhydride and pyridine to yield a monoacetate. One-proton double doublets at δ 4.74 (J=10 and 3.5 Hz) in the NMR spectrum of the monoacetate could be assigned to the acetoxy methine proton at C2. Thus, the structure of cotylenin I is represented as 4'−O-1,1-dimethylallylcotylenin (I). Recently we have reported the assignments of the carbon-13 NMR spectra of cotylenol and cotylenins A, C, E, F and I, which reasonably supported their proposed structures.6)

Cotylenin H exhibited a carbonyl absorption at 1730 cm⁻¹ in the IR spectrum. Its mass spectrum showed the molecular ion at m/e 524 and its dehydration ion at m/e 506, the latter corresponding to C_{29}H_{42}O_{8}. In addition, characteristic intense peaks were observed at m/e 327 (C_{17}H_{27}O_{6}), 287, 151 and 135, suggesting the presence of the sugar residue at C9 in the aglycone, cotylenol.5) The NMR spectrum (Table I) also indicated the presence of cotylenol and a sugar residue in the molecule. On acetylation with acetic anhydride and pyridine, it gave a partially acetylated product, diacetate. The NMR spectrum in d⁶-benzene displayed clearly separated signals of the sugar moiety protons; δ 5.66 (C_{6}'−H, dd, J=10 and 1 Hz), 5.41 (C_{4}'−H, dd, J=4.5 and 1 Hz), 5.21 (C_{1}'−H, d, J=4.5 Hz), 4.40 (C_{5}'−H, dt, J=10 and 3.5 Hz) and 3.46 (C_{8}'−H, d, J=3.5 Hz), indicating that the sugar of cotylenin H is 6-O-methyl-α-ribohexopyranosid-3-ulose.7) Cotylenin H was reduced with sodium borohydride in ethanol to yield an alcohol (V); NMR δ: 5.07 (C_{1}'−H, br.d, J=4 Hz) and ca. 4.1 (C_{5}'−H, m, which was coupled to C_{1}'−H with J=ca. 1 Hz). Its triacetate showed a characteristic fragmentation series of 6-O-methyl-triacetylhexopyranosyl at m/e 303, 243, 183, 141 and 109 in the mass spectrum.8) Expectedly, cotylenin H gave cotylenol by treatment with 1 N sodium hydroxide in ethanol-water.9) Thus, the structure of cotylenin H is assigned as that shown in II. The absolute configuration of the sugar part (D-glucopyranose) was deduced from the CD spectrum ([θ](nm): +230° (274)).

Cotylenin I has the same side chain, 1,1-dimethylallyl group, in the sugar part as that of fusicooccin10) and is a possible precursor at least for the major metabolites, cotylenins C and F which have a novel polyoxygenated isoprenoid unit at C4 in the sugar part. Cotylenin H is a diterpene glycoside having a 3-ketoglucopyranose which rarely occurs in nature. The sugar moiety of the principal metabolite, cotylenin A, is a 4'-ketocotylenin E, but not cotylenin I.

**EXPERIMENTAL**

Melting points were determined on a hot stage and are uncorrected. Optical rotations were determined on a JASCO DIP-4 polarimeter. IR spectra were measured with a JASCO IR-S spectrometer. The mass spectra were obtained with a Shimazu LKB 9000
GC-MS and a JEOLCO JMS-01SG mass spectrometer. NMR spectra were recorded on a Hitachi R-24 spectrometer. CD spectra were measured with a JASCO J-40A spectropolarimeter. Silica gel (Kanto Chemical Co., Inc.) and Kieselgel GF254 were used for column chromatography and preparative TLC (PTLC), respectively. Organic extracts were dried over anhydrous Na2SO4 and were concentrated by a vacuum rotary evaporator.

**Isolation of cotylenins I(1) and H(II)**

The EtOAc extracts of the culture filtrate (4.7 g) were chromatographed on silica gel with an acetonitrile-hexane mixture (1:4). Fractions between cotylenins A and C were concentrated and rechromatographed on silica gel with acetone-hexane (3:5) and then recrystallized from MeOH-H2O to give colorless needles (I), mp 91-94°C, [α]20D +31° (c=1.85, MeOH); IR νmax cm⁻¹: 3565, 1446, 1359, 1132, 1032 and 978; NMR: Table I; MS m/e (Calcd.): 594 (M+), 576.3657 (57%, C32H48O8 (576.3662), 507.2930 (20%, C28H42O8 (506.2879)), 327.1774 (28%, C19H25O (327.1807)), 269.1924 (28%, C19H27O2 (269.1905)), 211.1113 (43%, C15H23O (211.1123)) and 135.0849 (37%, C9H17O) (0.4ml) and dry pyridine (0.4ml) was kept at room temperature for 1 hr. The reaction mixture was diluted with water and extracted with CHCl3. The extracts were washed with water, dried and concentrated. The product was purified by PTLC with EtOAc–C6H6 (5:1) to yield the colorless monoacetate (26 mg); IR νmax cm⁻¹: 3610, 1783, 1760, 1750 (sh.), 1215 and 1201; MS m/e: 590 (M+–18), 411, 287 and 259; NMR δ (d6-benzene): 5.82 (d, J=2.5 Hz, C3–H), 5.66 (dd, J=10 and 1 Hz, C4–H), 5.41 (dd, J=4.5 and 1 Hz, C5–H), 5.21 (d, J=4.5 Hz, C6–H), 4.40 (dt, J=10 and 3.5 Hz, C7–H), 3.46 (d, J=3.5 Hz, C8–H) and 1.75 (s, OAc x 2).

**Cotylenin I monoacetate**

Cotylenin I (20 mg) was partially acetylated with acetic anhydride (0.5 ml) and dry pyridine (0.5 ml) at 5-10°C for 1 hr. The reaction mixture was diluted with ice-water and extracted with CHCl3. The extracts were washed with water, dried and concentrated. The product was purified by PTLC with EtOAc–C6H6 (1:1) to yield the colorless monoacetate (14 mg); MS m/e: 618 (M+–18), 439 and 287; IR νmax cm⁻¹: 3520, 1746 and 1238-1215 (br.); NMR δ (d6-acetone): 4.99 (d, J=3.5 Hz, C1′–H), 4.74 (dd, J=10 and 3.5 Hz, C2′–H) and 2.10 (s, OAc).

**Cotylenin H diacetate**

A mixture of cotylenin H (30 mg) in acetic anhydride (0.4 ml) and dry pyridine (0.4 ml) was kept at room temperature for 1 hr. The reaction mixture was diluted with ice-water and extracted with CHCl3. The extracts were washed with water, dried and concentrated. The product was purified by PTLC with acetone-hexane (2:3) to give the colorless diacetate (26 mg); IR νmax cm⁻¹: 3610, 1783, 1760, 1750 (sh.), 1215 and 1201; MS m/e: 590 (M+–18), 411, 287 and 259; NMR δ (d6-benzene): 5.82 (d, J=2.5 Hz, C3–H), 5.66 (dd, J=10 and 1 Hz, C4–H), 5.41 (dd, J=4.5 and 1 Hz, C5–H), 5.21 (d, J=4.5 Hz, C6–H), 4.40 (dt, J=10 and 3.5 Hz, C7–H), 3.46 (d, J=3.5 Hz, C8–H) and 1.75 (s, OAc x 2).

**Acid hydrolysis of cotylenin I**

A mixture of cotylenin I (10 mg) in MeOH (6 ml) and 0.005 N HCl solution (12 ml) was kept at 36°C for 8 hr. The reaction mixture was extracted with EtOAc. The extracts were washed with water, dried and concentrated. The major product was purified by PTLC with acetone-hexane (1:1) to give a colorless solid (4 mg), [α]25D +21° (c=0.18, MeOH).2) The IR spectrum was completely identical to that of cotylenin E (IV).

**Identification of the aglycone (III) of cotylenin I**

It was carried out by the same method as that for cotylenin F,1) from cotylenin I (20 mg) the aglycone (III, 3 mg, mp 156°C) was obtained.

**Alkaline treatment of cotylenin H**

A mixture of cotylenin H (10 mg) in EtOH (0.2 ml) and 2 N NaOH solution (0.2 ml) was stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with EtOAc. The extracts were washed with water, dried and concentrated. The product was purified by PTLC with EtOAc-hexane (1:1) and recrystallized from EtOAc-hexane to give colorless prism(III, 4 mg, mp 156-17°C).

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