Isolation and Identification of Cotylenins F and G[†]

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The structures of cotylenins F and G, isolated from the culture filtrate of a fungus (*Cladosporium* sp.), have been assigned as IV and V, respectively. Cotylenin G was derived from cotylenin F by treatment with a strong base.

Novel fungal glycosides, cotylenins, are new plant growth regulators, which were highly active in cotyledon assays.¹⁾ When calcium carbonate was added to the culture broth of the fungus (*Cladosporium* sp.) at its stationary phase, they were obtained in high yields.²⁾ In preceding papers, structures of cotylenol³⁾ and cotylenins A~E⁴⁾ were elucidated on the basis of chemical and spectroscopic evidence. Recently, we have isolated new related metabolites named cotylenins F and G from the culture filtrate. The present paper deals with their isolation and identification.

The culture filtrate²⁾ was extracted with ethyl acetate, and the concentrate obtained was carefully chromatographed on silica gel with chloroform-ethanol (100:8). Fractions between cotylenins C (I) and E (II) were rechromatographed on the same adsorbent with ethyl acetate-benzene (3:1). The less polar cotylenin was recrystallized from ethyl acetate to give colorless needles (cotylenin F (IV)), mp 178~9°C, $[\alpha]_D +16.4^\circ$. Another (cotylenin G (V)) was obtained as semisolid, $[\alpha]_D +27.2^\circ$.

Cotylenin F (IV), $C_{33}H_{54}O_{11}$, gave a positive sodium thiosulfate test. Its NMR spectrum (Table I) was essentially superimposable with that of I except the absence of the one-proton doublet (δ 4.78, $J=6.0$ Hz) observed in the latter. In the mass spectrum (m/e : 608 (M^+-18)), characteristic peaks were observed

at m/e 429, 287, 269 and 135, indicating the presence of the aglycone cotylenol (III) in the molecule and of the sugar residue at its C_9 .⁵⁾ Oxidation of IV with potassium periodate, followed by treatment with a strong base,⁶⁾ yielded the aglycone III, mp 156~7°C.³⁾ When IV was reduced with lithium aluminium hydride in tetrahydrofuran, it afforded a dihydroderivative (VI), mp 115~7°C, $C_{33}H_{56}O_{11}$. On prolonged acetylation with acetic anhydride and pyridine, VI gave a penta-acetate (VII). In the NMR spectrum, five acetoxyl and four secondary methyl resonances were observed; there were four acetoxy-methine protons in the region of δ 4.8~5.4, of which one was coupled to a new secondary methyl group (δ 1.15, $J=ca.$ 7 Hz) generated by the reduction of the epoxide function. From the above facts, it was concluded that IV was the partially reduced

[†] This paper constitutes Part V of Cotylenins, Leaf Growth Substances Produced by a Fungus. See Reference 4).

TABLE I. NMR SIGNALS OF I, IV AND V IN d⁶-ACETONE (60 MHz)

	C ₁ -H	C _{1'} -H	C _{1''} -H	OMe		C ₁₁ -Me	C _{2''} -Me	C ₁₅ -Me	C ₇ -Me
I	5.57 (d, 2.5) ^{a)}	4.99 (d, 3)	4.78 (d, 6)	3.29 3.37	2.4~ 3.1(m)	1.27	1.27	0.98 1.12	0.83
IV	5.56 (d, 2.5)	4.94 (d, 3)	—	3.31 3.37	2.6~ 3.0(m)	1.26	1.17	0.97 1.09	0.83
V	5.60 (d, 2.5)	4.97 ^{b)}	—	3.35 (6H)	—	1.26	1.04	0.95 1.11	0.84

^{a)} Coupling constant: Hz.^{b)} Diffused band, W_{h/2}=4 Hz.

product of the hemiacetal function of I to the corresponding primary alcohol grouping. This was confirmed by identification of VI and VII as the corresponding derivatives of I. Hence the structure of cotylenin F is expressed as IV.

Cotylenin G (V) gave a negative sodium thiosulfate test. And it was not oxidized with potassium periodate under the same conditions as those for IV. Its NMR spectrum (Table I) was quite similar to that of IV without multiplets of the epoxide ring protons. On acetylation, V readily gave a tetra-acetate (VIII). In the NMR spectrum, there were three protons of acylated shift in the region of δ 4.8~5.6, suggesting the presence of a primary hydroxyl group. The mass spectrum showed intense peaks at *m/e* 329, 287 and 269, which were also observed in that of III-diacetate. Treatment of V with 2,2-dimethoxypropane and a trace of *p*-toluene sulfonic acid in N,N-dimethylformamide afforded a mono-acetonide (IX) whose NMR spectrum exhibited signals of tertiary methyl groups of the acetonide at δ 1.37 and 1.46. Its monoacetate, mp 201~3°C, C₃₈H₆₀O₁₂, displayed two-proton multiplet at δ 4.8~5.2 in the NMR spectrum which were assigned to C₁'-H and C₂'-H. The mass spectrum (*m/e*: 693 (M⁺-15), 359 (sugar residue)) showed characteristic peaks at *m/e* 511, 287, 269, 151 and 135, indicating the presence of the aglycone III and of the sugar residue at its C₉.⁵⁾ From the above data, we inferred that the intramolecular epoxide cleavage by the hydroxyl group at C₃' in cotylenin F (IV) gave the isomeric cyclic ether, cotylenin G (V). This assumption was verified by derivation of V from IV with

a strong base. Thus, the structure of cotylenin G can be represented as V. Cotylenin G seems to be an artifact formed in the culture medium under mild alkaline conditions.

From the biosynthetic point of view, it is considered that cotylenin F could be the possible precursor for cotylenins C and A. Cotylenins C, E, F and G showed almost the same activity as that of the principal metabolite, cotylenin A, in a cucumber cotyledon assay.*

EXPERIMENTAL

Melting points are uncorrected. Unless otherwise stated, NMR spectra were recorded on a Hitachi R-24 and a JEOLCO JNM 4H-100 spectrometer in CDCl₃, and optical rotations were determined in MeOH on a JASCO DIP-4 polarimeter. IR spectra were measured with a JASCO IR-S spectrometer. The mass spectra were determined on a LKB 9000 GC-MS mass spectrometer. Kieselgel GF₂₅₄ was used for preparative TLC (PTLC). Organic extracts were dried over anhydrous Na₂SO₄ and concentrated by a vacuum rotary evaporator.

Isolation of Cotylenins F (IV) and G (V)

The EtOAc extracts of the culture filtrate (10 g) were chromatographed on silica gel with the mixed solvent (EtOH-CHCl₃; 4: 100 (cotylenin A), 8: 100 (I, IV, V) and 15: 100 (II)). Fractions containing IV were re-chromatographed on silica gel with EtOAc-benzene (3: 1) to give colorless crystalline substance (ca. 0.5 g). It was recrystallized from EtOAc to yield needles, mp 178~9°C, $[\alpha]_D^{20} + 16.4^\circ$ (*c*=1.19); IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3440, 1194, 1145, 1097, 1047, 903; NMR: Table I; MS *m/e*: 608 (M⁺-18), 593, 429, 287, 269, 135 (base peak). *Anal.* Found: C, 62.92; H, 8.68. Calcd. for C₃₃H₅₄O₁₁: C, 63.24; H, 8.68%. Fractions containing V were

* The details of biological activities will be disclosed elsewhere.

rechromatographed in the same manner as that of IV to give colorless semisolid (ca. 0.15 g), $[\alpha]_D^{20} + 27.2^\circ$ ($c=2.76$): attempts to crystallize it were unsuccessful. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3500, 1139, 1091, 1067, 1037, 917. NMR: Table I.

Identification of the aglycone (III) of IV

A mixture of IV (20 mg) in EtOH (1 ml) and KIO₄ (16 mg) in water (1 ml) was kept at 30°C for 12 hr. The reaction mixture was diluted with water and extracted with EtOAc (50 ml $\times$ 3). The EtOAc solutions were washed with water, dried and concentrated. The residue obtained was dissolved in EtOH (2 ml) and 1 N NaOH solution (2 ml). The mixture was stirred overnight at 30°C, then diluted with water and extracted with EtOAc (30 ml $\times$ 3). The EtOAc solutions were washed with water, dried and concentrated. The product was purified by PTLC with EtOAc-hexane (1:1) and recrystallized from EtOAc-hexane, mp 156~7°C. It was identified with an authentic sample of III by mp, mixed mp and IR spectrometry.

LiAlH₄ reduction of IV (VI)

A mixture of IV (50 mg) and an excess of LiAlH₄ in tetrahydrofuran (10 ml) was stirred at a room temperature for 1 hr. To decompose the excess reagent, EtOAc was added. The mixture was diluted with water and extracted with EtOAc (50 ml $\times$ 3). The EtOAc solutions were washed with water, dried and concentrated. The product was purified by PTLC with EtOAc-acetone (5:1) and recrystallized from EtOAc to yield colorless needles, mp 115~7°C, $[\alpha]_D^{20} + 10.7^\circ$ ($c=0.95$); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3510, 1191, 1082, 1040, 914. Anal. Found: C, 62.33; H, 8.71. Calcd. for C₃₃H₅₆O₁₁·1/2H₂O: C, 62.13; H, 9.01%. Its pentaacetate (VII); colorless semisolid; IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3550, 1735 (broad); NMR δ : 0.77, 1.03, 1.13, 1.15 (each 3H, d, $J=ca. 7$ Hz), 1.18, 1.22, 2.10, 3.38, 3.39 (each 3H, s), 2.02, 2.04 (each 6H, s), 4.82 (1H, dd, $J=3.5, 10$ Hz), 4.96 (1H, q, $J=ca. 7$ Hz), 5.27 (1H, dd, $J=4, 10$ Hz), 5.40 (1H, dd, $J=9, 10$ Hz); MS m/e : 820 (M^+-18), 778 (M^+-60).

Cotylenin G (V) tetra-acetate (IX)

IX was easily obtained by acetylation of V with acetic anhydride and dry pyridine; IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3550, 1753, 1742; NMR δ : 2.06 (6H, s), 2.12, 2.14 (each 3H, s), 4.9~5.7 (5H, m); m/e : 776 (M^+), 555, 403 (sugar residue), 343, 329, 287, 269, 151, 141, 135, 127.

Monoacetone of V (X)

A mixture of V (30 mg), 2,2-dimethoxypropane (1 ml) and a trace of *p*-toluene sulfonic acid in dimethyl

formamide (1 ml) was kept at a room temperature for 3.5 hr. The reaction mixture was diluted with 5% NaHCO₃ solution and extracted with EtOAc (30 ml $\times$ 3). The EtOAc solutions were washed with water, dried and concentrated. The product was purified by PTLC with EtOAc to yield amorphous solid (18 mg, X); NMR δ : 0.78, 0.95, 1.05, (each 3H, d, $J=ca. 7$ Hz), 1.20, 1.30, 1.37, 1.46, 3.36, 3.39 (each 3H, s), 5.05 (1H, b.s), 5.55 (1H, d, $J=2.5$ Hz). Its monoacetate; mp 201~3°C (EtOAc); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3550, 1757; NMR δ : 2.17, (3H, s), 4.8~5.2 (2H, m); MS m/e : 693 (M^+-15), 690 (M^+-18), 511, 359 (sugar residue), 314, 301, 299, 287, 282, 271, 185, 151, 141, 135, 111. Anal. Found: C, 64.25; H, 8.52. Calcd. for C₃₈H₆₀O₁₂: C, 64.38; H, 8.53%.

Conversion of IV to V

A mixture of IV (50 mg) in MeOH (3 ml) and 2 N KOH solution (30 ml) was kept at 30°C for 4 hr. The reaction mixture was extracted with EtOAc (30 ml $\times$ 3). The EtOAc solutions were washed with water, dried and concentrated. The product was purified by PTLC with EtOAc-acetone (5:1) to yield colorless semisolid (27 mg). It was identical with V in all respects.

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REFERENCES

- 1) T. Sassa, T. Tojyo and K. Munakata, *Nature*, **227**, 379 (1970); T. Sassa, *Agr. Biol. Chem.*, **34**, 1588 (1970); T. Sassa, *ibid.*, **35**, 1415 (1971).
- 2) T. Sassa, T. Negoro and H. Ueki, *ibid.*, **36**, 2281 (1972).
- 3) T. Sassa, *ibid.*, **36**, 2037 (1972); T. Sassa, A. Takahama and T. Shindo, *ibid.*, submitted for publication.
- 4) T. Sassa and A. Takahama, Symposium papers of 18th Symposium on the Chemistry of Natural Products, (Kyoto, 1974), p. 85; T. Sassa, M. Togashi and T. Kitaguchi, *Agr. Biol. Chem.*, submitted for publication.
- 5) T. Sassa, *ibid.*, **38**, 2041 (1974).
- 6) J. J. Dugan and P. de Mayo, *Canad. J. Chem.*, **43**, 2033 (1965).