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ROSIGENIN, AN UNUSUAL METABOLITE FROM *MYCOSPHAERELLA ROSIGENA*†

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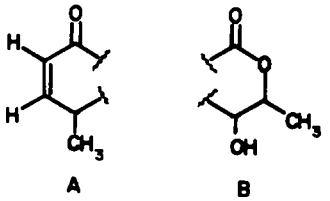
Abstract—A new metabolite, 3,6-dimethyl-4,10-dihydroxy-2-oxaspiro[4.5]dec-7-en-1,9-dione (1a), was isolated from a strain of *Mycosphaerella rosigena* grown on potato-agar medium. The structure was determined on the basis of chemical and spectroscopic evidence and confirmed by X-ray analysis.

In a previous paper[2] we reported the isolation of two new secondary metabolites mycochromone ($C_{16}H_{14}O_7$) and mycoxanthone ($C_{16}H_{12}O_6$) from the plant pathogenic fungus *Mycosphaerella rosigena*.[3] A study of the minor constituents of this fungus has now allowed the isolation of a new secondary metabolite (1a), for which we propose the name rosigenin ($C_{11}H_{14}O_5$) the present work deals with its structure determination.

RESULTS AND DISCUSSION

The fungus was grown on potato-agar medium and the metabolite extracted with ethyl acetate and purified by chromatography. The metabolite (1a) is a white solid, analysis and spectral data give formula $C_{11}H_{14}O_5$. The IR spectrum (3550 cm^{-1}, 1760 cm^{-1}, 1690 cm^{-1}) is consistent with the presence of OH groups (confirmed by the formation of the diacetate (1b), of a lactone group and of an unsaturated ketone. The ^1H NMR is fairly significant, as it shows a Me–$\dot{C}$H–CH=CH– system, three protons on carbons bearing an O atom (the first one of them at 4.35 δ is coupled with an OH and is shifted downfield by acetylation; the second also shifted by acetylation, is coupled with the third, which on its turn is further coupled with a Me group) and two OH groups, thus accounting for all the H atoms of the molecule. The ^{13}C NMR data are reported in Table 1 and show signals corresponding to eleven carbons.

Combination of all these data pointed to the partial structures A and B for 1a:

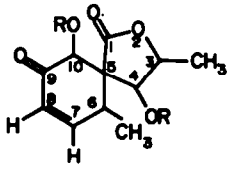


A B

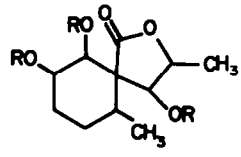
it remained to determine the exact location of the residual –$\dot{C}$H–OH group and of the quaternary carbon.

Useful information concerning the structure of rosigenin could be obtained from the hydrogenation in presence of PtO_2 which gave the tetrahydroderivative (2a). The absence in its IR spectrum of the band corresponding to the conj. CO and the presence in the ^1H NMR spectrum of a Me–$\dot{C}$H–CH_2–CH_2–CH(OH)–$\dot{C}$H–OH system clearly indicated that the α,β-unsaturated ketone was hydrogenated; acetylation of 2a gave a triacetate 2b which confirmed the presence of a new OH group in 2a with respect to 1a.

From these data it arises unequivocally that the –$\dot{C}$H OH in 1a is located α to the conj. CO and that the quaternary carbon must be the bridgehead between the two partial structures indicated above, so that the strucure of 1a is that shown.



1a R = H
1b R = Ac



2a R = H
2b R = Ac

A crystallographic determination confirmed the structure proposed for 1a and revealed the relative configuration of the substituent as given in Fig. 1. An ORTEP view of the molecule is shown in Fig. 2; the relevant bond lengths, bond angles and internal rotation angles are listed in Tables 2 and 3 respectively.

All the bond lengths are in the expected range[5] and all the angular deviations are readily explained taking into account steric hindrance.

The conformation of the cyclohexene moiety can be described in terms of the deviation from the least-square plane fitted to the C atoms: C(10) is pointing downward (–0.6 Å) while the remaining atoms are contained in the plane, their distances being in the range ±0.05 Å; the carboxy and the hydroxy groups are *trans* to each other (Fig. 1).

The shortest intermolecular contact (2.80 Å) is among atoms O(4) O(2) and the atoms related by the symmetry operators $\frac{1}{2}\pm x; -y; z\pm\frac{1}{2}$; all other distances being greater than 3.1 Å.

Rosigenin was essayed (50 ppm and 100 ppm in EtOH) on leaves of *Nicotiana tabacum* and *Beta vulgaris* and gave no phytotoxic effect.

The biosynthesis of rosigenin presents an unusually

†"Secondary mould metabolites" Part VIII. For Part VII (see Ref. [1]).

‡Centro del C.N.R. per le Sostanze Organiche Naturali.

Table 1. ^{13}C chemical shifts of rosigenin (1a) in DMSO

Carbon	δ	Mult.
Me-3 [a]	14.3	q
Me-6 [a]	17.9	q
C -6 [b]	33.0	d
C -5	59.5	s
C -4 [a]	73.3	d
C -10 [a]	75.4	d
C -3 [a]	79.0	d
C -8 [b]	124.3	d
C -7 [b]	152.0	d
C -1 [b]	175.5	s
C -9 [b]	196.9	s

a : the assignments were made by use of single-frequency selective heteronuclear decoupling (SFSD).

b : see ref. 4

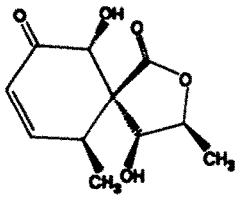


Fig. 1.

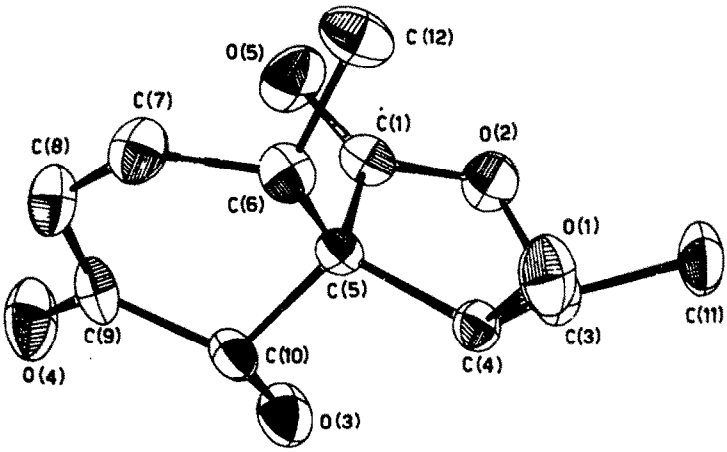


Fig. 2. An ORTEP[8] drawing of rosigenin (1a).

interesting problem. As is often the case with relatively small molecules, the structure of this C_{11} compound does not *a priori* suggest operation of any of the well-established biosynthetic sequences.

The carbon skeleton of rosigenin is identical with that of prephenic acid; however, the functionality of the molecule is not the one to be expected for a shikimate-derived structure. On the other hand, the carbon skeleton of rosigenin, with its quaternary C atom, could form from a poliketide chain only through some unusual rearrangement.

We hope to approach this problem experimentally by feeding of labelled substrates.

The structure of rosigenin (1a) suggests a biosynthetic relationship to the pentaketide-derived isocoumarins[6] (e.g. (4, R=H) and the *cis*-hydroxymellein[7] (5, R=H). It could conceivably be derived from an appropriately C_1-substituted mellein by a subsequent rearrangement. Alternatively it could possibly result from the rearrangement of an epimer of the isocoumarin precursor (3), having *cis*-fused rings which would therefore not be suitably orientated for the direct elimination of water required for isocoumarin formation.† An example of the 1,2-shift of a hydroxybenzoic acid carboxyl substituent has been recently described.[8]

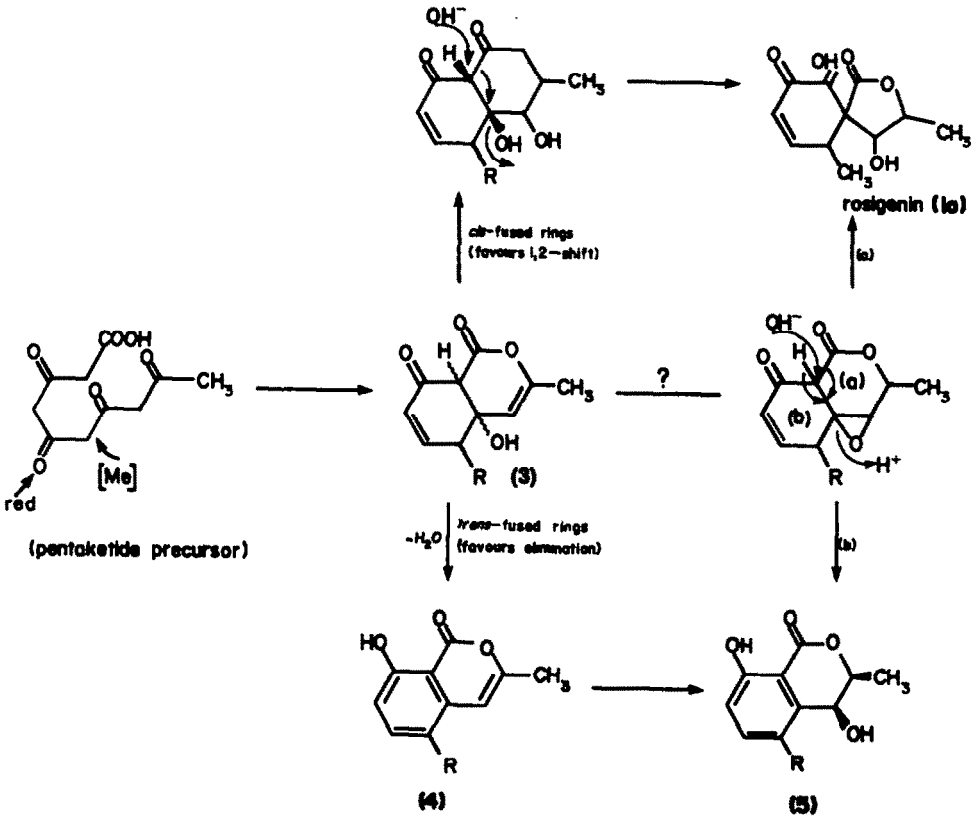
†We are indebted to the Referee for suggesting such a sequence: see Scheme 1.

Table 2. Bond lengths (Å) and angles (°) (e.s.d.'s for bond lengths in parentheses; e.s.d.'s on bond angles $\sigma = 0.2°$)

O(1)–C(4)	1.423(4)	O(1)–C(4)–C(3)	109.2
		O(1)–C(4)–C(5)	113.0
O(2)–C(1)	1.339(4)	O(2)–C(3)–C(4)	106.2
		O(2)–C(1)–C(5)	111.6
O(2)–C(3)	1.460(4)	O(2)–C(3)–C(11)	107.7
		O(3)–C(10)–C(5)	107.4
O(3)–C(10)	1.421(4)	O(3)–C(10)–C(9)	112.0
		O(4)–C(9)–C(8)	123.8
O(5)–C(1)	1.202(4)	O(4)–C(9)–C(10)	120.6
		O(5)–C(1)–O(2)	120.9
O(4)–C(9)	1.215(4)	O(5)–C(1)–C(5)	127.4
		C(1)–O(2)–C(3)	111.7
C(1)–C(5)	1.521(4)	C(1)–C(5)–C(4)	103.4
		C(1)–C(5)–C(10)	105.4
C(3)–C(4)	1.537(5)	C(3)–C(4)–C(5)	104.2
		C(4)–C(3)–C(11)	116.7
C(3)–C(11)	1.504(5)	C(4)–C(5)–C(6)	113.9
		C(5)–C(6)–C(7)	113.2
C(4)–C(5)	1.560(4)	C(5)–C(6)–C(12)	113.4
		C(5)–C(10)–C(9)	112.7
C(5)–C(6)	1.552(4)	C(6)–C(5)–C(10)	111.0
		C(6)–C(7)–C(8)	126.6
C(5)–C(10)	1.542(4)	C(7)–C(8)–C(9)	120.4
		C(7)–C(6)–C(12)	108.5
C(6)–C(7)	1.497(5)		
C(6)–C(12)	1.538(5)		
C(7)–C(8)	1.329(5)		
C(8)–C(9)	1.451(5)		
C(9)–C(10)	1.514(5)		

Table 3. Relevant internal rotation angles (°) (e.s.d.'s in parentheses)

O(1)–C(4)–C(3)–O(2)	104.0	(1)
O(2)–C(1)–C(5)–C(4)	-5.6	(1)
O(2)–C(3)–C(4)–C(5)	-16.9	(2)
O(3)–C(10)–C(9)–C(8)	165.9	(2)
O(4)–C(9)–C(8)–C(7)	166.3	(2)
O(5)–C(1)–O(2)–C(3)	173.4	(1)
C(1)–C(5)–C(4)–C(3)	13.6	(2)
C(1)–O(2)–C(3)–C(4)	14.5	(1)
C(1)–O(2)–C(3)–C(11)	140.3	(1)
C(3)–O(2)–C(1)–C(5)	-5.5	(2)
C(5)–C(6)–C(7)–C(8)	-5.4	(2)
C(5)–C(10)–C(9)–C(8)	44.5	(2)
C(6)–C(5)–C(10)–C(9)	-52.0	(2)
C(6)–C(7)–C(8)–C(9)	-3.8	(2)
C(8)–C(7)–C(6)–C(12)	-132.2	(2)
C(7)–C(6)–C(5)–C(10)	32.5	(1)
C(7)–C(8)–C(9)–C(10)	-16.3	(1)



Scheme 1.

EXPERIMENTAL

M.ps are uncorrected. NMR spectra were recorded with a Varian XL-100-15 spectrometer and with a Varian EM-390 spectrometer; the chemical shifts are given in ppm (δ) relative to internal Me_4Si. Column chromatography and tlc were performed with silica gel. Where not otherwise indicated the purity of the products was checked by tlc, NMR and MS and deemed sufficient for the purposes of structural elucidation.

Isolation and purification of the metabolite. A strain of *M. rosigena* 330.51 obtained from Centraal Bureau voor Schimmelcultures, Baarn, grown on potato-agar in Roux flasks was extracted twice with EtOAc after 3 weeks growth at room temp. The extracts were dried on Na_2SO_4 and evaporated to give a yellow-brown mixture of crude pigments. This mixture was adsorbed on the top of a chromatographic column and eluted with a mixture of hexane and EtOAc; from the less polar fractions mycochromone and mycoxanthone[2] were isolated, by using a ratio of hexane/EtOAc 1:1 the metabolite (1a) was isolated.

3,6 - *Dimethyl* - 4,10 - *dihydroxy* - 2 - *oxaspiro*[4.5]*dec* - 7 - *en* - 1,9 - *dione* (1a), white needles m.p. 171–172° (EtOAc). $[\alpha]_D^{25}$ −92.4° (*c* 0.22, MeOH). Mass 226, 208, 198, 182, 169, 151, 142, 135.

ν_{max} (KBr) cm^{-1}: 3550 (OH), 1760 (lactone CO), 1690 (conj. CO). (Found: C, 58.60; H, 6.15. $C_{11}H_{14}O_5$ requires: C, 58.40; H, 6.24%). ^{1}H-NMR (100 MHz, DMSO); δ 1.16 (3 H, d, J = 7.5 Hz, CH_3-6), 1.25 (3 H, d, J = 6.0 Hz, CH_3-3), 3.13 (1 H, m, H-6), 4.35 (1 H, d, J = 3.0 Hz, H-10), 4.60 (1 H, dd, J = 5.0 and 6.5 Hz, H-4), 4.70 (1 H, m, H-3), 5.70 (2 H, two OH), 5.94 (1 H, dd, J = 10.0 and 2.5 Hz, H-8), 6.63 (1 H, dd, J = 10.0 and 3.0 Hz, H-7).

3,6 - *Dimethyl* - 4,10 - *diacetoxy* - 2 - *oxaspiro*[4.5]*dec* - 7 - *en* - 1,9 - *dione* (1b). 50 mg of 1a in 1 ml dry pyridine and 1 ml Ac_2O were left 48 hr at 4°. The mixture was dissolved in $CHCl_3$ and treated with a sat $NaHCO_3$aq, water, sat $KHSO_4$aq, water and finally dried with Na_2SO_4. Plc (hexane/EtOAc, 1:1) gave a diacetate 1b, m.p. 132–133°. Mass 268 (M^+-42), 250 (M^+-60), 208, 179, 151, 135.

ν_{max} (Nujol) cm^{-1}: 1760 (CO), 1700 (conj. CO). ^{1}H NMR (100 MHz, $CDCl_3$): δ 1.34 (6 H, d, J = 6.0 Hz, two $\underline{CH_3}$–CH), 2.2 and 2.22 (6 H, s, two CH_3–CO), 2.98 (1 H, m, H-6), 4.78 (1 H, m, H-3), 5.52 (1 H, d, J = 6.0 Hz, H-4), 5.65 (1 H, s, H-10), 6.08 (1 H, dd, J = 10.0 and 2.5 Hz, H-8), 6.52 (1 H, dd, J = 10.0 and 3.0 Hz, H-7).

3,6 - *Dimethyl* - 4,9,10 - *trihydroxy* - 2 - *oxaspiro*[4.5]*decan* - 1 - *one* (2a). 30 mg of 1a in 10 ml MeOH were hydrogenated in presence of PtO_2 for 30 min at room temp. Evaporation of the solvent and plc (hexane/EtOAc 1:1) gave 25 mg of 2a, m.p. 220–221°. Mass 230, 212, 194, 155.

ν_{max} (KBr) cm^{-1}: 3420 (OH), 1712 (lactone CO). ^{1}H NMR (90 MHz, acetone-d_6): δ 1.05 (3 H, d, J = 6 Hz, $\underline{CH_3}$–CH), 1.35 (3 H, d, J = 6 Hz, $\underline{CH_3}$–CH), 1.4 – 2.0 (5 aliph. protons), 3.70 (1 H, d, J = 3 Hz, H-10), 3.9 (1 H, m, H-9), 4.7–5.0 (2 H, H-3 and H-4).

3,6 - *Dimethyl* - 4,9,10 - *triacetoxy* - 2 - *oxaspiro*[4.5]*decan* - 1 - *one* (2b). 20 mg of 2a in 0.5 ml dry pyridine and 0.5 ml Ac_2O were left 2 hr at room temp. The mixture was dissolved in $CHCl_3$ and worked up as described. The triacetate 2b has m.p. 124–125°. Mass 356, 296, 236, 194. ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.25 (6 H, d, J = 6 Hz, two $\underline{CH_3}$–CH), 1.4 – 1.20 (5 aliph. protons), 2.0, 2.02 and 2.12 (9 H, s, three CH_3–CO), 4.7 (1 H, m, H-3), 5.2 (2 H, H-9 and H-10), 5.45 (1 H, d, J = 4 Hz, H-4). ^{13}C NMR ($CDCl_3$): 170.4 – 169.6 (s, four CO), 76.9, 75.2, 72.4 and 67.7 (d, four CH–O), 53.9 (s, C-5), 30.9 (d, C-6), 26.3 and 24.5 (t, C-7, C-8), 20.7 and 20.4 (q, four CH_3–CO), 17.3 and 14.2 (q, CH_3-3 and CH_3-6).

X-*Ray crystallography*

An air stable white crystal of approximately 0.3 × 0.4 × 0.6 mm was used for the data collection.

Crystal data. $C_{11}O_5H_{14}$; orthorombic; a = 14.142(3); b = 10.746(2); c = 7.063(2) Å; (obtained by a least squares fit of the 2θ values of 25 reflections); V = 1073.36 Å^3; Z = 4; systematic absences indicated the space group $P2_12_12_1$.

Data were collected on a four circle Philips automated diffrac-

Table 4. Final positional and thermal parameters (*)

Atom	x/a	y/b	z/c	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{32}
O(1)	.3663(2)	.2642(2)	.7030(4)	2.8(1)	3.0(1)	5.1(1)	2.4(1)	-1.8(1)	0.6(2)
O(2)	.4029(2)	-.0304(2)	.7673(3)	3.1(1)	1.9(1)	2.8(1)	-1.3(1)	-0.7(1)	0.7(1)
O(3)	.6062(2)	.0311(2)	.6409(3)	2.1(1)	4.1(1)	3.0(1)	0.5(1)	0.3(1)	-2.3(2)
O(4)	.7294(2)	.0161(3)	.9339(4)	2.6(1)	4.4(1)	4.5(1)	3.0(2)	-2.0(2)	-1.4(2)
O(5)	.4894(2)	-.0468(2)	1.0296(2)	4.5(1)	2.6(1)	3.3(1)	-1.2(2)	-1.5(2)	2.4(2)
C(1)	.4646(2)	.0140(3)	.8943(4)	2.1(1)	1.7(1)	2.5(1)	-0.1(2)	0.8(2)	-0.2(2)
C(3)	.3937(2)	.0523(3)	.6043(4)	2.2(1)	3.2(1)	2.4(1)	-0.2(2)	-0.9(2)	-0.2(2)
C(4)	.4389(2)	.1765(3)	.6630(4)	1.8(1)	2.2(1)	2.3(1)	0.4(1)	-0.2(2)	1.1(2)
C(5)	.4995(2)	.1430(2)	.8403(4)	1.6(1)	1.4(1)	2.1(1)	0.07(1)	0.2(1)	0.5(1)
C(6)	.4908(2)	.2380(3)	1.0049(5)	2.4(1)	2.1(1)	3.0(1)	0.6(2)	0.3(2)	-0.7(2)
C(7)	.5736(3)	.2360(3)	1.1367(6)	3.5(1)	2.7(1)	2.7(1)	0.3(2)	-0.6(2)	-1.5(2)
C(8)	.6524(2)	.1700(3)	1.1164(5)	2.8(1)	3.1(1)	3.2(1)	-0.05(2)	-2.0(2)	-1.1(2)
C(9)	.6674(2)	.0952(3)	.9479(5)	1.5(1)	2.8(1)	3.6(1)	-0.1(1)	-1.0(2)	-0.3(4)
C(10)	.6037(2)	.1254(3)	.7818(4)	1.5(1)	2.8(1)	2.7(1)	-0.07(2)	0.3(2)	0.5(2)
C(11)	.2910(3)	.0558(4)	.5494(7)	2.5(1)	4.9(2)	4.1(2)	-1.5(3)	-2.7(3)	0.7(4)
C(12)	.4000(3)	.2224(4)	1.1221(6)	3.0(1)	4.1(2)	4.3(1)	0.9(3)	2.8(3)	-0.4(3)

(*)e.s.d.s in parentheses.

Anisotropic thermal factors in the form :

$T = \exp -1/4(B_{11}a^{*2}h^2+B_{22}b^{*2}k^2+B_{33}c^{*2}l^2+2B_{12}a^*b^*hk+2B_{13}a^*c^*hl+2B_{23}b^*c^*kl)$

tometer. Reflections were measured using a $\omega/2\theta$ technique up to $\theta_{max} = 25.0°$ (graphite monochromated MoKα radiation).

The scan speed was 0.04° s^{-1} and the scan width 1.20°. Two background counts were measured each side of the peaks and the values averaged. The intensities of three standard reflections were monitored every 60 min to check the stability of the crystal and of the experimental conditions; no significant variations were detected.

1800 reflections were collected, of which 1011 were considered as observed having net intensities $I \geq 3\sigma(I)$. The data have been corrected for Lorentz and polarization but no absorption or extinction corrections were found necessary.

The structure was solved with MULTAN 74.[9] An E map revealed all but the H atoms.

The refinement was carried out by least squares using anisotropic thermal factors for C and O atoms; at the end of the refinement the contribution from the H atoms (in their calculated positions) were added but not refined. The scattering factors from Ref. [10] were used.

The final R factor was 0.053 for the observed reflections (weighted R = 0.084). The final atomic coordinates are given in Table 4.†

†A list of observed and calculated structure factors can be obtained from the authors.

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