Metabolites of the higher fungi. Part 32.[1] Rosnecatrone, a phytotoxic bicyclo[4.1.0]hept-3-en-2-one from the fungus *Rosellinia necatrix* Prill

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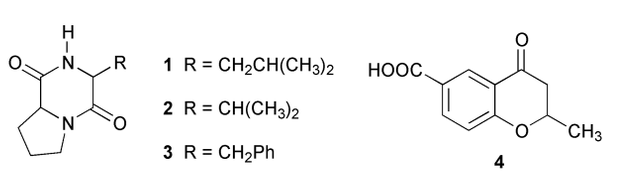
Received (in Cambridge, UK) 11th October 2000, Accepted 17th January 2001
First published as an Advance Article on the web 8th February 2001

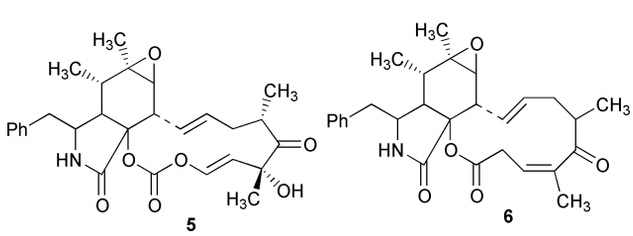
Rosnecatrone **7** is a phytotoxic metabolite isolated from cultures of a virulent strain of the fungus *Rosellinia necatrix*. The compound is identified as 5-hydroxy-4-hydroxymethyl-1-(1-hydroxy-3-methylbut-2-enyl)-3-[(*E*)-propenyl]-7-oxabicyclo[4.1.0]hept-3-en-2-one by chemical and physical methods. The absolute configuration is determined and the X-ray structure of its borohydride reduced derivative, rosnecatrol **10** as the tetra-acetate is described.

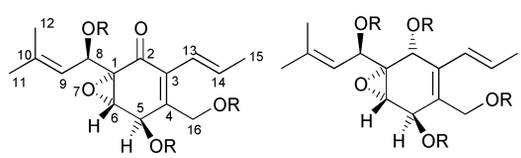
The fungus family Xylariaceae comprises a large number of genera each containing a large number of species. One of these genera, the genus *Rosellinia*, contains a number of important plant pathogens. Typically they cause canker or root rot and are most prevalent in regions where relatively high temperatures tend to occur. Typical pathogenic species are exemplified by *R. pepo* found on herbaceous woody species in the tropics, *R. acuta* on the roots of tea, *R. bunodes* on the roots of coffee, *R. desmazieresii* on the roots of *Salix repens*, and *R. necatrix* on the roots of many types of fruit trees, grape vines and tea bushes.

Results and discussion

Examination of the metabolites produced by individual species has been confined to *R. desmazieresii*, which has been reported[2] to produce cytochalasin E, but in our hands has produced only cytochalasin D, and *R. necatrix*, where all the compounds are reported as either plant growth inhibitors or are capable of causing typical disease symptoms associated with the species. These compounds comprise the diketopiperazines **1**, **2** and **3** and the chromanone rosellinic acid **4** from a strain found growing on tea[3,4] in Japan, cytochalasin E **5** from a strain of unreported origin[5,6] and rosellichalasin **6** from a Japanese strain.[7] In Europe *R. necatrix* is prevalent especially in the Mediterranean and closely adjacent countries and during an examination of strains collected in Portugal from the roots of apple, walnut, pear, cherry and poplar trees it became clear that there was considerable variation in their metabolite producing capabilities. Strains collected from apple and walnut in the Alcobaca region produced small quantities of cytochalasin E. However, a strain from cherry and poplar in the Alcobaca and Corinbra regions, respectively, produced significantly higher yields of cytochalasin E, and one strain from pear in the Tomar region of central Portugal gave a substantial yield of a new phytotoxic 7-oxabicyclo[4.1.0]hept-3-en-2-one **7** which we







name rosnecatrone. The compound is produced in the medium and cytochalasin E is only a minor metabolite from this strain. All the other Portuguese strains examined produced only trace amounts of this new compound. This is a very virulent strain of this fungus and initial tests have shown that the compound is more phytotoxic than cytochalasin E when applied to apple and geranium seedlings.

Rosnecatrone **7** was isolated when the fungus was surface cultured on a 3% malt medium containing 6% added glucose. The metabolite was detected by TLC as a bright pink colouration, which develops at room temperature over 30 min with an acetic acid : anisaldehyde : sulfuric acid (98 : 1 : 1) spray reagent. The other metabolites do not show under these conditions; cytochalasin E and any other metabolites show only after the plate has been heated at 110 °C for 2–3 min. The absence of any other colouration produced in the cold allows the ready detection of this compound in cultures of strains producing only trace amounts.

Solvent extraction of the culture medium gave a gum which after chromatography in the solvent system chloroform : methanol (95 : 5) gave a fraction yielding rosnecatrone **7**, as

DOI: 10.1039/b008195g

Table 1 ^{1}H and ^{13}C NMR data for rosnecatrone in $CDCl_3$

Position	δ_H	δ_C	$\delta_C{}^2J_{C-H}$	$\delta_C{}^3J_{C-H}$
1		60.61		
2		195.62		
3		131.35		
4		147.74		
5	4.91 (1 H, d, *J* 1.47)	64.21		131.35, 60.61
6	3.83 (1 H, d, *J* 1.47)	57.89		147.74
8	5.06 (1 H, d, *J* 8.8)	65.47		
9	5.16 (1 H, ddd, *J* 8.8, 1.1, 1.1)	120.31		
10		139.31		
11	1.75 (3 H, d, *J* 1.1)	25.93	139.31	120.31, 18.74
12	1.73 (3 H, d, *J* 1.1)	18.74	139.31	120.31, 25.93
13	6.04 (1 H, dd, *J* 15.8, 1.1)	121.72		
14	5.91 (1 H, dq, *J* 15.8, 6.23)	135.06		
15	1.85 (3 H, dd, *J* 6.23, 1.1)	19.25	135.06	121.92
16a	4.44 (1 H, d, *J* 14.6)	62.12		131.35
16b	4.68 (1 H, d, *J* 14.6)			

silky colourless needles mp 103 °C from benzene–hexane, $[\alpha]_D^{20}$ −276 (*c* 0.19 in EtOH), ν_{max}(KBr)/cm^{-1} 3380 and 1680; λ_{max}(EtOH)/nm 272 (ε/dm^3 mol^{-1} cm^{-1} 2968). The NMR data are summarised in Table 1.

In the ^{13}C NMR spectrum the fifteen carbons comprise three methyl groups at δ 18.74, 19.25 and 25.93, one methylene at δ 62.12, six methine at δ 57.89, 64.21, 65.47, 120.31, 121.72 and 135.06, and five quaternary carbons at δ 60.61, 131.35, 139.31, 147.74 and 195.62. Three of the five oxygen atoms in **7** can be assigned to hydroxy groups by the formation of a gummy triacetate **8**, $C_{21}H_{26}O_8$, with acetic anhydride and pyridine, and a fourth to the carbonyl of an unsaturated ketone identified by the quaternary carbon at δ 195.62 and the IR absorption at 1680 cm^{-1}. The five carbons between δ 50–70 are attached to four oxygen atoms and this means that two share a common oxygen and this suggests the presence of an ether or epoxide linkage.

In the ^{1}H NMR spectra two coupled single proton doublets at δ 4.44 and 4.68 (*J* 14.6 Hz) move to δ 4.79 and 4.86 on acetylation. This shift ($\Delta\delta$ 0.27 on average) identifies them as primary alcohol methylene protons. This conclusion is confirmed by the ^{13}C–^{1}H COSY spectrum, where there is a correlation to a methylene carbon attached to oxygen at δ 62.12; sub-unit **a**. Similarly two single proton doublets at δ 4.91 and

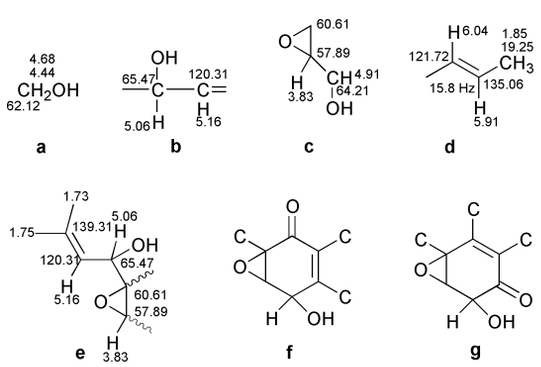


Chart 1 Sub-units **a** to **g**.

5.06 independently correlate to the methine carbons attached to oxygen at δ 64.21 and 65.47, and move on acetylation to δ 6.05 ($\Delta\delta$ 1.0) identifying them as methine protons of two secondary alcohol groups. In the ^{1}H–^{1}H COSY spectrum the secondary proton at δ 5.06 is coupled (*J* 8.8 Hz) to an olefinic proton at δ 5.16 associated with the unsaturated methine carbon at δ 120.31; sub-unit **b**. The other secondary proton at δ 4.91 is coupled to a methine proton at δ 3.83 (*J* 1.47 Hz) which is attached to the oxygen bearing methine carbon at δ 57.89. The position of this latter proton is unaffected by acetylation so this

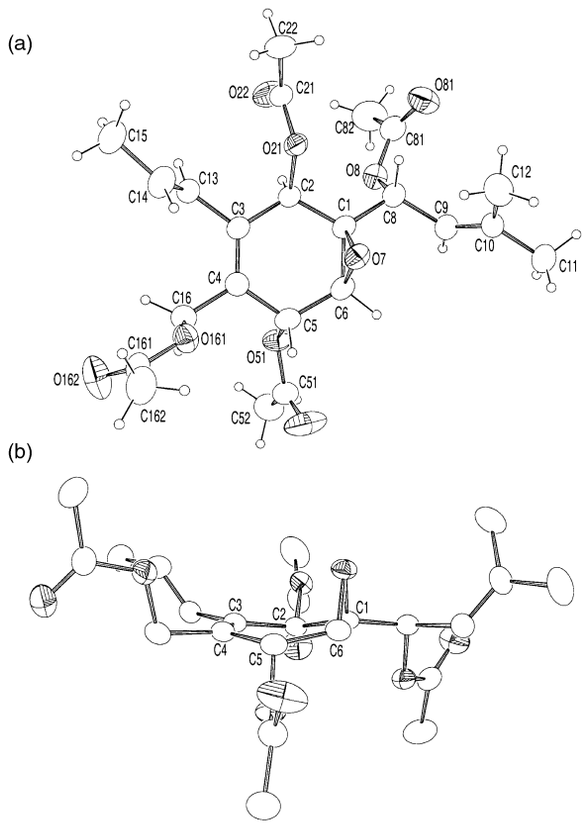


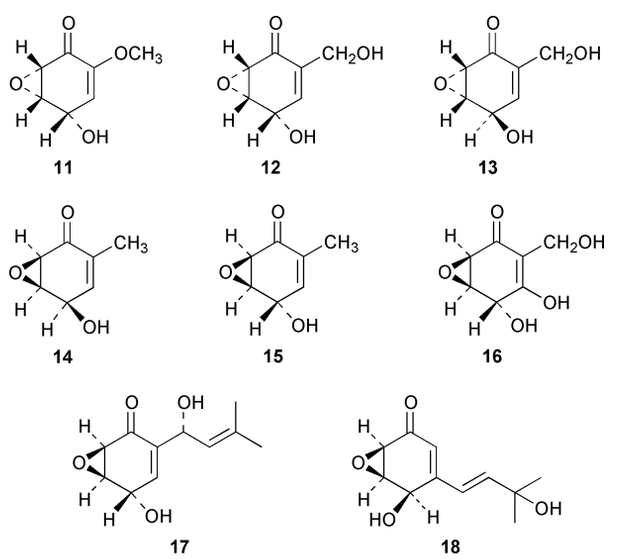
Fig. 1 Views of the crystal structure of rosnecatrol tetra-acetate **10** showing (a) the numbering scheme adopted and (b) the disposition of the ring substituents and the relative stereochemistry of the chiral centres in the molecule.

oxygen and methine carbon must be involved in the ether/epoxide linkage, the other side of which must be occupied by the oxygen bearing quaternary carbon at δ 60.61, sub-unit **c**. Also in the ^{1}H–^{1}H COSY spectrum the methyl doublet at δ 1.85 couples to an olefinic single proton doublet of quartets at δ 5.91 which is also *trans* coupled (*J* 15.8 Hz) to an olefinic proton doublet at δ 6.04. These protons correlate to the methine carbons at δ 135.06 and δ 121.72, respectively and constitute the propenyl sub-unit **d**. Three olefinic quaternary, one olefinic methine and two methyl carbons remain to be assigned. In the long range ^{13}C–^{1}H 2D correlation spectrum obtained by using the FLOCK pulse sequence[22] the proton signals of the two methyl groups at δ 1.73 and 1.75 correlate with the unsaturated quaternary carbon at δ 139.31, and also the unsaturated methine carbon at δ 120.31. The proton signal for the latter at δ 5.16 is coupled to the secondary alcohol methine proton at δ 5.06, which sees the quaternary carbon of the epoxide ring at δ 60.61 thus establishing the connection to sub-unit **c**, and hence sub-unit **e**. The absence of any additional coupling to the hydroxymethyl protons, and to the 13-H proton of the propenyl group, indicates these are attached to the two remaining unsaturated quaternary carbons and leads to two possible cyclic structures **f** and **g**.

Reduction of rosnecatrone with sodium borohydride gave a crystalline tetrahydroxy compound, rosnecatrol **9**, which gave a crystalline tetra-acetate **10** with acetic anhydride and pyridine. In rosnecatrol the new secondary methine proton at δ 5.60 is a singlet establishing the cyclic structure of the parent ketone as **f**. The tetraacetate gave crystals suitable for X-ray structure analysis; the structure of **10** with the atom numbering scheme is shown in Fig. 1. The six-membered ring is distorted from planarity with C(2) and C(5) lying significantly above the least-squares plane due to the slight boat conformation adopted. The structure indicates positions of the pentenyl and propenyl groups on opposite sides of the six-membered ring

and establishes the relative stereochemistry at the C-1, C-2, C-5, C-6 and C-8 stereocentres. The molecule crystallises in the chiral space group *P*1 and the hand identified in the specimen studied is 1*R*, 2*R*, 5*R*, 6*S*, 8*R*. However, although the Flack *x* parameter of 0.2(3) appears not to differ significantly from zero for this hand, its relatively large esd precludes confident assignment of absolute structure. Bond lengths in the molecule lie within expected ranges and the double bonds are clearly identified by shorter bond lengths: the relatively short C(3)–(4) distance is consistent with the presence of a double bond in the ring opposite the epoxide and the short C(9)–C(10) distance confirms the position of the double bond of the pentenyl substituent at C(1). The propenyl substituent at C(3) has the C(13)–C(14) double bond in the *E*-configuration. The crystal packing appears to be largely dominated by van der Waals interactions and long-distance interactions between methyl protons and carbonyl oxygens. However, two relatively short contacts corresponding to weak CH···O hydrogen bonds are apparent. The 5-acetoxy carbonyl and the methine proton at C(13) are separated by 2.530(5) Å corresponding to the propagation of a chain parallel to the *a*-axis of the crystal; the 2-acetoxy carbonyl and the methine proton at C(6) are separated by 2.479(4) Å, consistent with "cross-linking" the chains parallel to the *b*-axis.

Rosnecatrone is a new member of a group of fungal bicyclo[4.1.0]heptenones which comprises chaloxone **11** from *Chalara microspora*,[8] epoxydon **12** from a *phoma* species,[9] isoepoxydon **13** from *Penicillium urticine*[10] and *Poronia punctatum*,[11] the desoxyepiepoxydon isomers **14** and **15** from *Penicillium patulum*[12] and *P. claviforme*,[13] terremutin **16** from *Aspergillus terreus*,[14] panepoxydon **17** and isopanepoxydon **18**



from several *Panus* species[15] and coriloxin **19** from *Coriolus vernicipes*[16] and in the case of the compounds **11**–**18** the inverse octant rule[17] has been used to assign their absolute configuration from their circular dichroism (CD) spectra. The rule states that in compounds containing the 7-oxabicyclo[4.1.0]hept-3-en-2-one (5,6-epoxycyclohex-2-enone) structure, the n→π* transition (R band) in the CD spectrum has a sign dictated by the octant in which the oxa oxygen atom lies. In the case of rosnecatrone the Cotton curve (Fig. 2(a)) is negative at 350 nm which indicates that of the eight possible stereoisomers only four structures will have the 7-oxa-2-one moiety configuration, as in **h** and **i** (Fig. 3). This compares with terremutin and panepoxydon, which also show a negative Cotton effect at 318 and 341 nm respectively. However, this makes no distinction between the (−)-*cis* and (−)-*trans* isomers nor the a-OH or e-OH conformers. This can be resolved from the sign of the 3-en-2-one chromophore at 270 nm (K band). In this case the sign is negative as in terremutin and panepoxydon and this

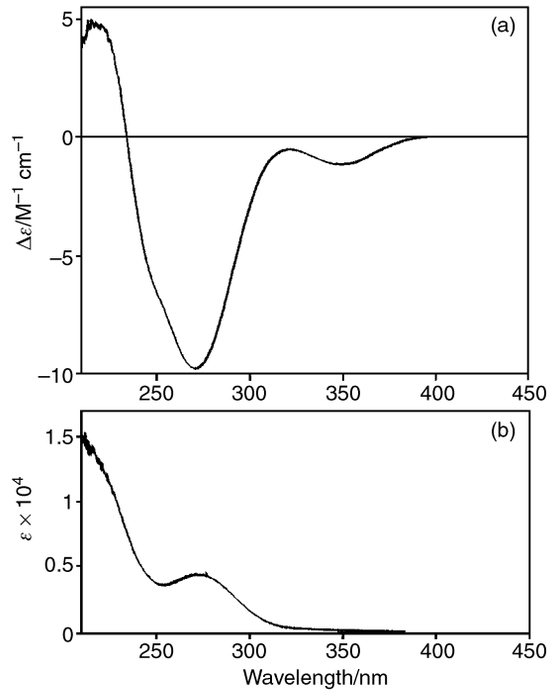


Fig. 2 CD (a) and UV (b) spectra of rosnecatrone **7**.

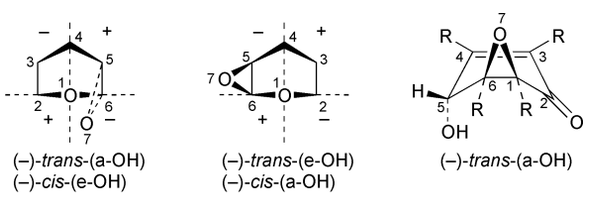


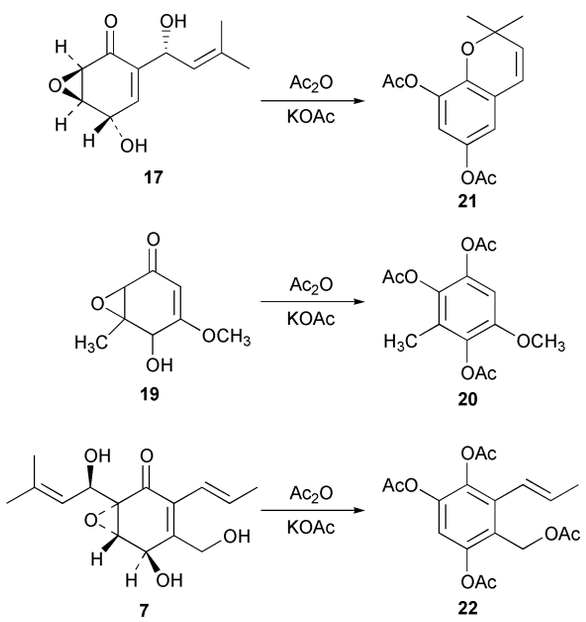
Fig. 3

means that only the two structures in **h** are now possible. The X-ray diffraction data identify the configuration of the C-5 hydroxy and the C-6 oxa oxygen as *trans* and the absolute configuration and conformation of rosnecatrone is therefore that of the (−)-*trans* (a-OH) structure as in **j** (Fig. 3). The configuration of rosnecatrone at all the enantiomeric centres can now be assigned as 1*S*, 5*S*, 6*R* and 8*S*. The same conclusion regarding the *trans* configuration at C-5 and C-6 can be obtained from the small coupling constant between the protons at these positions (*J* 1.47 Hz) and the judicious use of the Karplus equation.

Rosnecatrone is more substituted than any of the compounds **11**–**19**, and although the hydroxymethyl group is to be found in epoxydon **12** and isoepoxydon **13**, and the hydroxypentenyl group in panepoxydon **17** and its closely related analogues, only in rosnecatrone **7** and coriloxin **19** is a substituent found on one of the epoxide ring positions. This causes significant differences in their chemistry compared with other members of the series. Coriloxin and rosnecatrone respectively produce the expected mono- and triacetate with acetic anhydride and pyridine, but the others rearrange to benzenoid derivatives. However, coriloxin can be made to aromatise and in our hands has given the aromatic triacetate **20** in quantitative yields when heated with acetic anhydride and potassium acetate. Panepoxydon is reported to aromatise, rearrange in the side chain, and cyclise to the chromene **21** with this reagent mixture (Scheme 1). Under similar conditions rosnecatrone does not undergo cyclisation, instead aromatisation occurs with extrusion of the hydroxypentenyl side chain and the formation of a crystalline tetraacetate, $C_{16}H_{20}O_6$, **22**. In the ^{13}C NMR spectrum of this compound, only the hydroxymethylene carbon remains in the δ 50–70 region. The six aromatic and the two unsaturated

Table 2 Comparison of SpecInfo calculated and actual ^{13}C NMR data for the aromatic rings of 1,4,5-triacetoxy- and 1,4,6-triacetoxy-3-acetoxymethyl-2-(prop-1-enyl)benzenes

Carbon No.	1,4,5-Triacetoxy isomer—δ_C Calc.	1,4,6-Triacetoxy isomer—δ_C Calc.	Actual δ_C
C-1	154.55	142.53	142.90
C-2	127.37	127.37	135.44
C-3	126.38	126.38	124.36
C-4	144.93	148.75	147.56
C-5	132.38	110.55	116.32
C-6	110.55	132.38	137.88

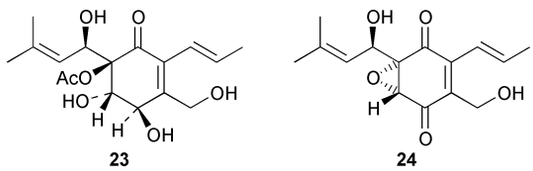


Scheme 1

propenyl carbons occur between δ 116–148; four quaternary carbons at δ 167–170 associated with four new methyl carbons at δ 19–21 identify it as a tetra-acetate. In the ^1H NMR spectrum an aromatic proton resonance occurs as a singlet at δ 6.97 and the signals for the retained propenyl side chain and hydroxymethyl group appear as in rosnecatrone. The formation of this aromatic acetate can be envisaged as the result of a retro aldol reaction leading to the loss of the hydroxypentenyl side chain.

Two isomers are possible, the product depending on the course of the final elimination of the acetoxy groups resulting from the opening of the epoxide ring. In the case of the epoxydons **12** and **13**, terremutin **16** and panepoxydon **17** the acetoxy group introduced into the aromatic ring is located on the carbon adjacent to the original carbonyl group. Rosnecatrone upon aromatisation appears to reflect this pattern and yields **22**. This was established using SpecInfo[18] to predict the ^{13}C NMR spectrum of each isomer (Table 2). When the predicted shifts are compared with the experimental values, the 6-acetoxy isomer clearly shows the best correlation.

Rosnecatrone is decomposed immediately by mineral acid to a complex mixture of products. Substantial decomposition takes place in boiling acetic acid over 20 min, but in this case a low yield (8%) of a gummy mono-acetate **23**, resulting from an opening of the oxirane ring, is obtained. The acetoxy group would be expected to enter at the most substituted carbon atom and this is supported by the unchanged resonance position of H-6; if an acetoxy group had been introduced at that position a substantial downfield shift of this proton would have been expected. In addition a configurational inversion will be expected at C-1. A naturally occurring mono-acetate from a *Phylosticta* species[19] shows similar structural features and



presumably originates from a biological nucleophilic attack by acetate on epoxydon. Oxidation of **7** with MnO_2 yields the epoxy-1,4-benzoquinone **24**, which is identified in the ^1H NMR spectrum by the absence of any coupling at the C-6 position, and in the ^{13}C NMR spectrum by the loss of the C-5 methine carbon at δ 64.20 and its replacement by an additional carbon at δ 194.56.

The simple cyclohex-2-enones **11**–**16** and **19** presumably have a tetraketide origin as has been shown in the case of epoxydon **12** by feeding experiments. Interestingly Turner[20] in his book has assigned the prenylated panepoxydon **17** and its closely related analogues to the chapter dealing with the metabolites derived *via* the shikimic acid pathway because of their structural similarity to the postulated intermediates in the biosynthesis of the benzofurans from *Stereum subpileatum*.[21]

Experimental

General

Mps were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on either a Perkin-Elmer 681 or a Nicolet 205 spectrophotometer; mass spectra (EI) and (FAB using 3-nitrobenzyl alcohol as matrix) were measured on an AEI MS 902 spectrometer equipped with an MSS Data System for Windows (Data Version 2.03, Software Version 10.0). Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. Extracts were dried over Na_2SO_4. All chromatography columns, thick layer (PLC) and thin layer (TLC) glass plates were made up using Merck Kieselgel GF_{254} Column sizes and solvent systems used are specified in each case.

^1H and ^{13}C NMR spectra, using tetramethylsilane as an internal standard, were determined at 270 and 67.8 MHz respectively with a JEOL GX270 spectrometer fitted with a dual 5 mm C/H probe. ^1H NMR spectra were acquired with 32K data points over a spectrum width of 3001.2 or 6002.4 Hz; *J* values are given in Hz. Carbon atom types were established in the ^{13}C NMR spectrum by employing a combination of broad-band proton-decoupled and distortionless enhancement by polarisation transfer (DEPT) experiments with 32K data points over a spectrum width of 17 605.6 Hz. Assignments were established by employing a combination of 1-D and 2-D NMR experiments. Two-dimensional spectra were acquired and processed by standard JEOL software; ^1H–^1H correlations by double quantum-filtered COSY (VDQFN), resolution 2.93 Hz in the $f1$ and $f2$ domains, PW1 = PW2 = $\pi/2$; [$^1J_{C-H}$] ^{13}C–^1H correlations (VCHSHF), resolution $f2$ 17.19 Hz and $f1$ 5.9 Hz, pulse delay 1, 2 or 3 s, J_{C-H} 140 Hz; and [$^2J_{C-H}$ and $^3J_{C-H}$] ^{13}C–^1H correlations were established using the FLOCK pulse sequence of Reynolds *et al.*,[22] resolution $f2$ 17.19 Hz and $f1$ 5.9 Hz, pulse delay 1, 2 or 3 s, Δ^1 86.5 ms and Δ^2 46.5 ms or Δ^1 44.0 ms and Δ^2 24.0 ms.

Crystal structure determination †

A colourless block of rosnecatrol tetra-acetate **10** (0.477 × 0.347 × 0.347 mm^3) was mounted on a glass fibre and data were collected in the θ-range 4.7 to 60° using Cu-Kα radiation on a Stoe STADI-4 four-circle diffractometer.

† CCDC reference number 152459. See http://www.rsc.org/suppdata/p1/b0/b008195g/ for crystallographic data in CIF or other electronic format.

Crystal data. $C_{23}H_{30}O_9$, $M = 450.5$, triclinic, $a = 8.3738(17)$, $b = 8.5966(17)$, $c = 10.291(2)$ Å, $\alpha = 67.40(3)$, $\beta = 75.08(3)$, $\gamma = 67.33(3)°$, $U = 625.9(2)$ Å^3, $T = 295$ K, space group $P\bar{1}$ (no. 1), $Z = 1$, μ(Cu-Kα) = 0.771 mm^{-1}, 2112 reflections measured, 2108 unique ($R_{int} = 0.039$) which were used in all calculations. The final $R1$ [$I > 2\sigma(I)$] = 0.0347 and $wR2$ (all data) = 0.0979.

Isolation of rosnecatrone

Rosellinia necatrix was surface cultured at 25 °C in subdued daylight for eight weeks on a 3% malt extract medium containing 6% added glucose in Thompson bottles (8 × 2 dm^3), each bottle containing 1 dm^3 of medium. The medium became pale yellow during the growth. The thin brittle grey mycelia were uniformly black on the lower surface. The medium was filtered through muslin and extracted three times with ethyl acetate, the extract dried (Na_2SO_4) and evaporated to yield a brown gum (5.3 g) which was applied to four PLC silica plates (20 × 100 cm). Development with the solvent system chloroform : methanol (95 : 5) gave a gummy fraction (pink colouration with anisaldehyde spray reagent), which crystallised from a benzene–hexane mixture to yield *5-hydroxy-4-hydroxymethyl-1-(1-hydroxy-3-methylbut-2-enyl)-3-[(E)-propenyl]-3-oxabicyclo-[4.1.0]hept-3-en-2-one*, *rosnecatrone* **7**, as silky needles (3.2 g), mp 103 °C, *m/z* 280 (Found: C, 64.2; H, 7.0. $C_{15}H_{20}O_5$ requires C, 64.3; H, 7.2%).

Rosnecatrone triacetate

A mixture of rosnecatrone (100 mg), acetic anhydride (3 cm^3) and pyridine was set aside overnight. The mixture was poured into water (25 cm^3) and set aside overnight. The opaque solution was extracted with diethyl ether (×3), the ether washed with sodium hydrogen carbonate solution, then water and dried. Evaporation yielded *rosnecatrone triacetate* **8** as an amber coloured gum (110 mg); *m/z* (EI) 406.16043. $C_{21}H_{26}O_8$ requires 406.16278; ν_{max}($CHCl_3$/cm^{-1}) 1743 and 1687; δ_H($CDCl_3$) 1.75 (3 H, d, J 1.1, 11-H_3), 1.85 (3 H, d, J 4.76, 15-H_3), 1.86 (3 H, s, 12-H_3), 2.03 (3 H, s, OAc), 2.08 (3 H, s, OAc), 2.12 (3 H, s, OAc), 3.77 (1 H, d, J 2.2, 6-H), 4.79 (1 H, d, J 13.8, 16-H_a), 4.86 (1 H, d, J 13.8, 16-H_b), 5.11 (1 H, ddd, J 9.3, 1.1 and 1.1, 9-H), 5.95–6.15 (3 H, m 5-H, 13-H and 14-H) and 6.24 (1 H, d, J 9.3, 8-H); δ_C($CDCl_3$) 18.92 (C-12), 19.31 (C-15), [20.70, 20.77, 21.04 (OAc)], 25.99 (C-11), 56.36 (C-6), 60.90 (C-1), 61.52 (C-16), 65.23 (C-5), 66.12 (C-8), 117.21 (C-9), 121.59 (C-13), 136.21 (C-3 and C-10), 136.59 (C-14), 141.28 (C-4), [169.58, 170.17, 170.39 (OAc)] and 192.99 (C-2).

Sodium borohydride reduction of rosnecatrone to rosnecatrol

Sodium borohydride (60 mg) was added to a solution of rosnecatrone (200 mg) in aqueous methanol (4 cm^3, 50%) and the mixture stirred for 1 h. The methanol was removed under vacuum at room temperature and the aqueous solution (2 cm^3) acidified (H_2SO_4, M). The solution was extracted with ethyl acetate (×3) and the extract washed, dried and evaporated to yield a foamy gum which crystallised from ethyl acetate to yield *rosnecatrol* **9**, *2,5-dihydroxy-4-hydroxymethyl-1-(1-hydroxy-3-methylbut-2-enyl)-3-(prop-1-enyl)-7-oxabicyclo[4.1.0]hept-3-ene*, as needles (30 mg), mp 176–180 °C; *m/z* 282 (Found: C, 63.7; H, 7.5. $C_{15}H_{22}O_5$ requires C, 63.8; H, 7.85%); δ_H(C_5D_5N) 1.65 (3 H, s, 12-H_3), 1.69 (3 H, dd, J 6.6 and 1.5, 15-H_3), 1.71 (3 H, d, J 1.1, 11-H_3), 4.07 (1 H, d, J 1.47, 6-H), 4.85 (1 H, d, J 12.1, 16-H_a), 4.95 (1 H, d, J 12.1, 16-H_b), 5.34 (1 H, s, 5-H or 2-H), 5.38 (1 H, d, J 8.4, 8-C), 5.60 (1 H, s, 2-H or 5-H), 5.73 (1 H, dd, J 8.4 and 1.1, 9-H), 6.29 (1 H, dq, J 15.76 and 6.6, 14-H) and 6.66 (1 H, d, J 15.76, 13-H); δ_C(C_5D_5N) 18.70 (C-12), 19.07 (C-15), 25.99 (C-11), 59.65 (C-6), 60.23 (C-16), 64.68 (C-1), 65.42 (C-5), 65.82 (C-8), 70.03 (C-2), 125.03 (C-9), 127.76 (C-14), 129.61 (C-13), 133.46 (C-4*), 133.98 (C-10), C-3 lies under pyridine signal at 135.84. * Could be interchanged with C-3.

A solution of the above reduction product (30 mg) in acetic anhydride (0.5 cm^3) and pyridine (1 drop) was set aside overnight at room temperature. The mixture was poured into water (3 cm^3), and this solution evaporated to dryness under reduced pressure. The residual solid was dissolved in ethyl acetate, the solution was washed once with water, dried and evaporated to give a gum, which crystallised from hexane to yield *rosnecatrol tetra-acetate* **10** as clusters of chunky needles (23 mg), mp 103 °C (Found: C, 61.2; H, 6.6. $C_{23}H_{30}O_9$ requires C, 61.3; H, 6.7%); ν_{max}($CHCl_3$)/cm^{-1} 1743 and 1739.

1,4,6-Triacetoxy-3-acetoxymethyl-2-(prop-1-enyl)benzene 22

A mixture of rosnecatrone (200 mg) and anhydrous potassium acetate (100 mg) in acetic anhydride (3 cm^3) was refluxed for 10 min. The yellow mixture was cooled, poured into water and the solution set aside overnight at 5 °C. The turbid solution was extracted with ethyl acetate (×4), the extract washed with water, dried and the solvent evaporated. PLC of the gummy residue in the solvent system toluene : ethyl acetate : acetic acid (50 : 49 : 1) gave a fraction R_f 0.85 (turning pink then orange with the anisaldehyde reagent after heating), which crystallised from hexane to yield *1,4,6-triacetoxy-3-acetoxymethyl-2-(prop-1-enyl)benzene* as aggregates of needles (27 mg), mp 106 °C (Found: C, 59.2; H, 5.1. $C_{18}H_{20}O_8$ requires C, 59.3; H, 5.5%); ν_{max}(KBr)/cm^{-1} 1768 and 1737; δ_H($CDCl_3$) 1.88 (3 H, dd, J 6.59 and 1.64, 9-H_3), 2.04 (3 H, s, OAc), 2.26 (3 H, s, OAc), 2.27 (3 H, s, OAc), 2.30 (3 H, s, OAc), 5.07 (2 H, s, 10-H_2), 5.93 (1 H, dq, J 6.59 and 16.12, 8-H), 6.27 (1 H, dd, J 16.12 and 1.64, 7-H), 6.97 (1 H, s, 5-H); δ_C($CDCl_3$) [19.12, 20.40, 20.69 and 20.83 (OAc)], 58.23 (C-10), 116.32 (C-5), 122.11 (C-8), 124.36 (C-3), 134.63 (C-7), 135.44 (C-2), 137.88 (C-6), 142.90 (C-1), 147.56 (C-4) and [167.76, 167.99, 168.95, 170.61 (OAc)].

Action of acetic acid on rosnecatrone

A solution of rosnecatrone (100 mg) in acetic acid (7 cm^3) was refluxed for 20 min. The brown solution was rotary evaporated at 40 °C and the oily residue purified by PLC using the solvent system toluene : ethyl acetate : acetic acid (50 : 49 : 1). The mixture comprised unchanged rosnecatrone R_f 0.35 and three other components R_f 0.8, 0.65 and 0.55. The component R_f 0.55 (red colouration with anisaldehyde spray reagent) was eluted to yield *4,5-dihydroxy-3-hydroxymethyl-6-acetoxy-6-(1-hydroxy-3-methylbut-2-enyl)-2-(prop-1-enyl)cyclohex-2-en-1-one* **23** as a pale yellow gum (6 mg), ν_{max}($CHCl_3$/cm^{-1}) 1744 and 1688; δ_H($CDCl_3$) 1.72 (3 H, d, J 1.10, 11-H_3), 1.74 (3 H, d, J 1.23, 12-H_3), 1.85 (3 H, d, J 5.1, 14-H_3), 2.10 (3 H, s, 6-OAc), 3.82 (1 H, d, J 1.8, 5-H), 4.72 (1 H, d, J 1.8, 4-H), 4.82 (1 H, d, J 13.4, 15H_a), 4.98 (1 H, d, J 13.4, 15-H_b), 5.02 (1 H, d, J 8.6, 7-H), 5.15 (1 H, d, J 8.6, H-8) and 6.05 (2 H, m, H-12 and 13); δ_C($CDCl_3$) 18.74 (C-11), 19.31 (C-14), 20.88 (6-OAc), 25.91 (C-10), 58.21 (C-5), 61.07 (C-6), 62.48 (C-15), 64.62 (C-4), 65.99 (C-7), 120.69 (C-8), 121.70 (C-12), 134.31 (C-2), 135.96 (C-13), 139.25 (C-9), 142.22 (C-3), 171.20 (6-OAc) and 195.71 (C-1).

Oxidation of rosnecatrone

To a solution of rosnecatrone (200 mg) in dry benzene (4 cm^3) was added MnO_2 (200 mg). The mixture was stirred 5 min, then more MnO_2 added (200 mg) and then more again (200 mg) after a further 25 min. The mixture was stirred for a further 1.3 h and the solution filtered through Celite and evaporated to yield an orange brown gum (157 mg), which after PLC in the solvent system chloroform : methanol (95 : 5) gave *4-hydroxymethyl-1-(1-hydroxy-3-methylbut-2-enyl)-3-[(E)-propenyl]-7-oxabicyclo[4.1.0]hept-3-ene-2,5-dione* **24** as an orange gum (51 mg), ν_{max}($CHCl_3$)/cm^{-1} 1690, running as the fastest component

on the plate at approximately double the speed of the original and developing a pink colouration much more slowly than the original with the anisaldehyde spray reagent; $\delta_H(CDCl_3)$ 1.76 (3 H, d, *J* 1.1, 12-H_3), 1.78 (3 H, d, *J* 1.1, 11-H_3), 1.94 (3 H, dd, *J* 6.6 and 1.1, 15-H_3), 2.65 (2 H, br s, OH), 3.93 (1 H, s, 6-H), 4.39 (1 H, d, *J* 12.82, 16-H_a), 4.54 (1 H, d, *J* 12.82, 16-H_b), 5.01 (1 H, d, *J* 8.79, 8-H), 5.20 (1 H, dd, *J* 8.79 and 1.1, 9-H), 6.34 (1 H, dd, *J* 15.76 and 1.1, 13-H) and 6.51 (1 H, dq, *J* 15.76 and 6.6, H-14); $\delta_C(CDCl_3)$ 18.74 (C-12), 20.07 (C-15), 25.91 (C-11), 56.50 (C-6), 56.98 (C-16), 63.02 (C-1), 64.77 (C-8), 120.60 (C-9), 121.96 (C-13), 136.76 (C-4), 140.04 (C-10), 141.98 (C-14), 142.44 (C-3), 194.51 (C-5) and 194.56 (C-2).

Acknowledgements

We wish to acknowledge the use of Chemical Concepts SpecInfo Database at the EPSRC Chemical Database Service at Daresbury,[18] and the EPSRC Chiroptical Spectroscopy Service, Kings College London for the CD measurement. Cultures of the fungus (R10) are maintained at ENFVN, Alcobaca and at the University of Bradford.

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