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Phytochemistry, Vol. 21, No. 7, pp. 1818–1819, 1982.
Printed in Great Britain.

0031-9422/82/071818-02$03.00/0

TWO NEW ISOPRENOID SPIRO COMPOUNDS FROM POTATO TUBERS INFECTED WITH *PHOMA EXIGUA*

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(*Received* 9 *September* 1981)

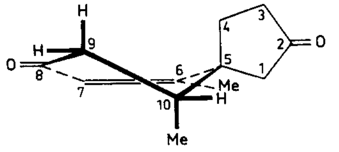
Key Word Index—*Solanum tuberosum*; Solanaceae; potato; *Phoma exigua* var. *foveata*; isoprenoids; 6, 10-dimethylspiro [4,5] dec-6-ene-2, 8-dione; 2-(1′, 2′-dihydroxy-1′-methylethyl)-6, 10-dimethyl-9-hydroxy-spiro-[4,5] dec-6-en-8-one.

Abstract—Two new isoprenoid compounds, 6, 10-dimethylspiro [4,5] dec-6-ene-2, 8-dione and 2-(1′, 2′-dihydroxy-1′-methylethyl)-6, 10-dimethyl-9-hydroxyspiro [4,5] dec-6-en-8-one, have been isolated from potato tubers infected with *Phoma exigua* var. *foveata*.

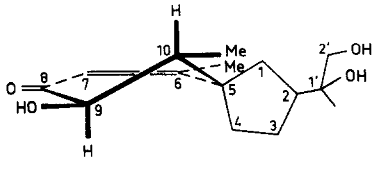
Coumarins and sesquiterpenes in the protective zone produced by potato tubers infected with the fungus *Phoma exigua* var. *foveata* have been the subject of a previous study [1]. Two new isoprenoids, **1** and **2**, from the same source have now been isolated and identified.

In its ^{1}H NMR spectrum, compound **1** showed a pattern for a cyclohexenone ring similar to that of compound **3** reported previously [1]. According to the ^{13}C NMR spectrum it contained, together with the signals from the cyclohexenone ring, an additional carbonyl signal (δ 216.7) and three triplets indicating a cyclopentanone ring. The presence of a cyclopentanone was also confirmed by an additional absorption from a carbonyl group at 1740 cm^{-1} in the IR spectrum. High-resolution mass spectrometry gave the molecular formula $C_{12}H_{16}O_2$. On the basis of these spectral data, compound **1** was given the structure 6, 10-dimethylspiro [4,5] dec-6-ene-2, 8-dione.

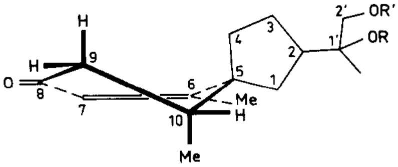
Strong similarities were noted between the data for compound **2** and the corresponding data for **3**. Significant differences in the ^{1}H NMR spectrum were noted only for H–9 and Me–10. The H–9 appeared as a doublet at δ 3.83, and the Me–10 was shifted downfield 0.2 ppm compared with the signals in the spectrum of **3**. In the ^{13}C NMR spectrum of **2**, C–9 and C–10 were shifted downfield (δ 31.3 and 8.6, respectively) while Me–10 was shifted upfield (δ 3.6) compared with the corresponding data of **3**. These data, and the mass spectrum showing M^{+} at *m/z* 268 (1.7 and 5.1% rel. int. at 70 and 20 eV, respectively) indicated hydroxyl substitution at C–9. The conformation of the cyclohexenone ring in **2** was fixed by the coupling constant (12.7 Hz) between the protons H–9 and H–10, corresponding to an a–a orientation of the two protons [2]. Hence, the structure of compound **2** is 2-(1′,2′-dihydroxy-1′-methylethyl)-6, 10-dimethyl-9-hydroxyspiro-[4, 5]-dec-6-en-



1



2



3 R = R' = H

3a R, R' = CMe$_2$

8-one. Apart from the pseudo-diequatorial orientation of the substituents at C-9 and C-10, the stereochemistry is not known.

The conformation of the cyclohexenone ring in **1** could not be investigated because of the small amount of compound isolated. Therefore, the isopropylidene derivative (**3a**) of **3** was used as a model compound. The similarities between compounds **1** and **3** in structure and in the shift (δ 2.72 and 2.67) and vicinal coupling constant (5.6 and 5.0 Hz) for one of the two protons at C-9 in ^{1}H NMR spectrum make it likely that the two compounds are in the same conformation. The use of shift reagents on **3** was not successful, but the ^{1}H NMR spectrum of **3a** in the presence of Eu(fod)$_3$ and with decoupling of the methyl group attached to C-10 clearly showed the ABX pattern for the protons attached to C-9 and C-10 ($J_{AX} = 4.4$ Hz, $J_{BX} = 4.7$ Hz). This determines the conformation of the cyclohexenone ring in compound **3a** with the methyl group at C-10 in an axial orientation. It is suggested that this also applies to compounds **1** and **3**. Other features of their stereochemistry are not known.

EXPERIMENTAL

Extraction and isolation. After extraction with petrol, the extract described in the previous paper [1] was made slightly basic (pH ~ 8) with NaHCO$_3$ and further extracted with CH$_2$Cl$_2$. The extract was evaporated and fractionated on Si gel, using CHCl$_3$–EtOH with increasing EtOH content (5–50%) as eluent. It gave 13 fractions. Fraction 4 was rechromatographed on Si gel with C$_6$H$_{12}$–EtOAc (2:1) as eluent, giving 1.4 mg of pure **1**. Fraction 11 was rechromatographed on Si gel using CHCl$_3$–EtOH (15:1) as eluent and finally on Sephasorb (ultra fine) with H$_2$O–EtOH (3:1) as eluent, giving 4.7 mg of pure **2**.

Compound **1**. Amorphous, $[\alpha]_D^{23} - 11°$ (MeOH; c 0.14). ^{1}H NMR (90 MHz, CDCl$_3$): δ 5.87 (1 H, *br q*, H-7), 2.72 (1 H, *dd*, $J_{9,9} = 17.3$ Hz, $J_{9,10} = 5.6$ Hz, H-9), 1.94 (3H, *d*, $J = 1.4$ Hz, Me-6), 1.0–2.5 (8H, *m*), 1.01 (3H, *d*, $J = 6.6$ Hz, Me-10). ^{13}C NMR (22.53 MHz, CDCl$_3$): δ 216.7 (*s*, C-2), 197.6 (*s*, C-8), 163.4 (*s*, C-6), 126.9 (*d*, C-7), 48.0 (*s*, C-5), 42.0 (*t*, C-9), 39.0 (*d*, C-10), 45.8, 36.6 and 33.2 (*t*, C-1, C-3 and C-4), 21.5 (*q*, Me-6), 16.6 (*q*, Me-10). GC/MS, 70 eV, m/z (rel. int.): 192 [M$^+$] (43), 164 (27), 150 (100), 122 (65), 108 (78), 93 (65), 79 (74). M$^+$; obs. 192.1151, calc. for C$_{12}$H$_{16}$O$_2$: 192.1150. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1665. UV λ_{max}^{MeOH} nm: 236.

Compound **2**. Amorphous, $[\alpha]_D^{23}$ 88.7° (MeOH, c 0.15). ^{1}H NMR (90 MHz, CDCl$_3$): δ 5.84 (1 H, *br q*, H-7), 3.83 (1 H, *d*, $J_{9a,10a} = 12.7$ Hz, H-9), 3.59 (1H, *d*, $J_{2',2'} = 11.4$ Hz, H-2'), 3.36 (1 H, *d*, $J_{2',2'} = 11.4$ Hz, H-2'), 2.06 (3 H, *d*, $J = 1.1$ Hz, Me-6), 1.3–2.4 (8 H, *m*) 1.23 (3 H, *s*, Me-1'), 1.18 (3H, *d*, $J = 5.3$ Hz, Me-10). ^{13}C NMR (22.53 MHz, CDCl$_3$): δ 199.3 (*s*, C-8), 172.6 (*s*, C-6), 121.9 (*d*, C-7), 74.1 (*d*, C-9), 73.4 (*s*, C-1'), 69.6 (*t*, C-2'), 51.7 (*s*, C-5), 48.1 (*d*, C-10), 47.3 (*d*, C-2), 36.2 (*t*, C-1), 31.0 (*t*, C-4), 28.8 (*t*, C-3), 22.6, 22.4 (*q*, Me-1' and Me-6), 12.3 (*q*, Me-10). MS, 70 eV, m/z (rel. int.): 268 [M]$^+$ (1.7), 267 (3.4), 250 (4.7), 237 (11), 219 (13), 210 (36), 192 (29), 175 (23), 161 (53), MS, 20 eV, m/z (rel. int.): 268 [M]$^+$ (5.1), 267 (13), 250 (9.4), 237 (25), 219 (29), 210 (77), 192 (65), 175 (52), 161 (100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480 (*br*), 1675, 1620. UV λ_{max}^{MeOH} nm: 240.

Compound **3a**. Compound **3** (50 mg) was converted to the 1', 2'-isopropylidene derivative (**3a**) [3]. The product was purified on a short Si gel column (CHCl$_3$–EtOH, 10:1) and **3a** was obtained as an oil (55 mg). $[\alpha]_D^{23} - 87.5°$ (MeOH; c 2.50).

^{1}H NMR (90 MHz, CDCl$_3$): δ 5.75 (1 H, *br q*, H-7), 3.87 (1 H, *d*, $J = 8.4$ Hz, H-2'), 3.75 (1 H, *d*, $J = 8.4$ Hz, H-2'), 2.68 (1 H, *dd*, $J_{9,9} = 17.1$ Hz, $J_{9,10} = 5.1$ Hz, H-9), 1.5–2.3 (9 H, *m*), 1.95 (3 H, *d*, $J = 1.3$ Hz, Me-6), 1.40 (6 H, *s*, CMe$_2$), 1.31 (3 H, *s*, H-1'), 0.97 (3 H, *d*, $J = 6.7$ Hz, Me-10). MS, 70 eV, m/z (rel. int.): 292 [M]$^+$ (4), 277 (11), 217 (13), 178 (15), 137 (23), 115 (100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1660, 1610. UV λ_{max}^{MeOH} nm: 242.

Acknowledgements—I thank Professor Olof Theander for his interest in this work, Docent Kjell Olsson for valuable discussions, and Mr Rolf Andersson for recording and assisting in interpreting the NMR spectra.

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