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Acetylenes and dichloroanisoles from *Psathyrella scobinacea*

Ahmed A. Taha *

Department of Chemistry, College of Science, University of Bahrain, Isa Town, PO Box 32038, Bahrain

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Abstract

The Et_2O extract from *Psathyrella scobinacea* culture fluids contained three new acetylenic alcohols: deca-5,7,9-triynol, (−)hepta-4,6-diyne-2,3-diol, and (−)hept-*cis* 4-en-6-yne-2,3-diol; two known dichloroanisoles: 3,5-dichloro-4-methoxybenzaldehyde and 3,5-dichloro-4-methoxybenzyl alcohol; and three known acetylenic acids: octa-2,4,6-triynoic acid, dec-*trans*-2-ene-4,6,8-triynoic acid and its *cis*-isomer.

Keywords: *Psathyrella scobinacea*; Coprinaceae; White-rot fungi; Isolation; C_{10} and C_7 acetylenic alcohols; Acetylenic acids; Dichloroanisoles

1. Introduction

In our search for new chemical products from basidiomycetes (Ahmed et al., 1982) we have described previously the isolation of the monoterpene lactone, scobinolide, from the culture fluids of the white-rot fungus *Psathyrella scobinacea* (Gadir et al., 1986). White-rot fungi are a group of basidiomycetes that can degrade lignin in forest-litter and as such contribute to environment clean up. When grown on synthetic culture media some of these fungi have the ability to degrade organic pollutants (Bergbauer et al., 1997). We now describe further the isolation of new and known acetylenic, aromatic and chloroaromatic metabolites not reported before in this fungus. Members of the genus *Psathyrella*, such as *Psathyrella (Drosophila) subatrata* (Fr.), were found previously to produce the acetylenes Drosophilins C and D (Jones et al., 1960) and the antibiotic mycomysin (Harvey et al., 1952). The same fungus was also found to produce chloroanisole metabolites e.g. antibiotic Drosophilin A (Anchel, 1952). Anisaldehyde itself is a common constituent of higher plants and was reported as a metabolite of several basidiomycetes (Turner, 1971). Recently more chloroanisole metabolites have been isolated from other basidiomycetes, e.g. from *Bjakandra* sp. and *Phellinus fastuosus* (Field et al., 1996, 1997). Chloroanisole compounds produced by white-rot fungi are considered to play a role, perhaps as catalytic cofactors (Field and Teunissen, 1998), in the enzymic degradation of lignin, a property which make these fungi useful in a number of ways, e.g. in biopulping processes (Brennan, 1998).

2. Results and discussion

Analysis of the culture fluids of *P. scobinacea* revealed the presence of three acetylenic acids and four acetylenic alcohols. The acetylenic acids were the known octa-2,4,6-triynoic acid (**1**), the ubiquitous dehydromatricaria acid (**2**) and its *cis*-isomer (**3**). The alcohols are the triynols (**6** and **7**) and the two new acetylenic C7-diols named scobinynediol-I (**8**) and-II (**9**). To our knowledge a limited number of C7-acetylenic alcohols have been isolated before from fungi, e.g. hepta-5,7-diyn-3-ol from *Gemnopilus spectabilis* (Hearn et al., 1973). The two isomeric triynols isolated from *P. scobinacea* were deca-5,7,9-triynol (**6**) and deca-4,6,8-triynol (**7**) both new as fungal metabolites; triynol (**7**), however, has been isolated before from *Lactuca pulmieri* (lettuce) (Bently et al., 1969) and from *Tridax trilobata* (Bohlmann et al., 1970). Furthermore, we have isolated from the same fungus two chlorinated anisoles, namely the 3,5-dichloroanisaldehyde (**4**) and the 3,5-dichloroanisyl alcohol (**5**). The structures of these fungal metabolites were established by ^{1}H NMR, EIMS, CIMS, UV, IR, mp, bp, TLC, and polarimetry methods; and by comparison with data reported in the literature.

* Tel.: +973-2-782-343; fax: +973-782-329.
E-mail address: ahmedabd@sci.uob.bh

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These compounds were isolated as follows: The concentrated Et_2O extract of the culture fluids (35 l) was separated into neutral and acid fractions (saturated $NaHCO_3$ solution), the acid fraction esterified (MeOH–H_2SO_4) and the methyl esters (500 mg) were separated by column chromatography using silica gel as adsorbent. The fractions were eluted according to increasing polarity and identified as follows.

Compound **1** methyl ester gave a molecular ion $[M]^+$ in the CI-mass spectrum at *m/z* 146 (88%) and a base peak at 115 (M^+-OMe) consistent with the molecular formula $C_9H_6O_2$. The compound showed a UV spectrum typical of a triyne ester (327 nm) whereas its IR spectrum indicated the presence of disubstituted acetylene (2240 cm^{-1}) and an ester carbonyl (1735 cm^{-1}). The ^1H NMR spectrum showed two singlets at $\delta = 2.0$ (*s*, H-1) and $\delta = 3.8$ (*s*, H-2) with an integration ratio of 1:1 due to a propyne methyl and an ester methyl groups, respectively. The triyne acid (**1**) has been reported previously in the fungal species of *Psilocybe merdaria, Kuehnermyces mutabilis, Russula vesca and Ramaria flava* (Hearn et al., 1973).

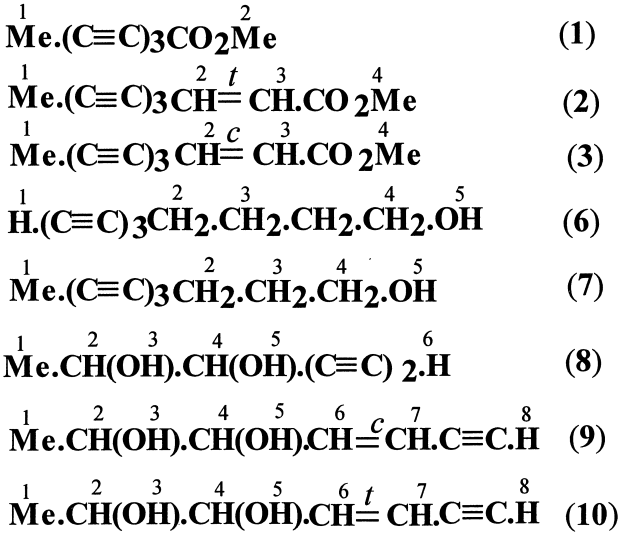
Compound **2** methyl ester was isolated and gave a molecular ion (M^+) in the CI-mass spectrum at *m/z* 172 (100%) consistent with the molecular formula $C_{11}H_8O_2$ whereas fragments at *m/z* 141 and 113 were due to loss of –OMe and $–CO_2Me$, respectively. UV (Et_2O) absorptions at 344, 320, 301, 283, 268, 255, and 243 nm were typical of a triynene ester. The structure was substantiated by the presence of IR absorptions at 2239 (C≡C stretching), 1725 (ester carbonyl) and 950 (*trans* CH=CH) cm^{-1}; and by ^1H NMR resonances at $\delta = 2.0$ (*s*, 3H, H-1) and at $\delta = 3.80$ (*s*, 3H, H-4) similar to those in **1** above; whereas signals at $\delta = 6.80$ (*d*, $J = 18$, H-2) and 6.35 (*d*, $J = 18$, H-3) were due to protons of a *trans* double bond. The ester had identical melting point (104–106°) and spectral data to those reported for dehydromarticaria ester (Gardner et al., 1960).

The culture fluids of this fungus also contained another triynene acid (**3**) isolated in minute quantities (5 mg) as the methyl ester. The material was not enough to permit IR or NMR analysis but its melting point [113–114°C; lit. mp 112–115°, (Bell et al., 1956)], UV absorption (345 nm) and mass spectrum fragmentation pattern data: *m/z* 172 [M^+ 100%], *m/z* 141 (loss of-OCH_3) and *m/z* 113 (loss of $–CO_2CH_3$) were identical to those quoted (Gardner et al., 1960) for the methyl ester of deca-*cis*-2-ene-4,6,8-triynoic acid (**3**), the *cis*-isomer of (**2**). Both *cis* and *trans* compounds (**2** and **3**) are common in plants but the *trans*-isomer is more frequent in fungi (Bu'Lock, 1964).

A mixture of minute quantities of esters was isolated from the mother liquor of the acid fraction from which compound **4a** methyl ester was separated and identified by GC–MS. The fragmentation pattern at *m/z* 234 (M^+, 42%) with base peak at *m/z* 203 (loss of methyl group) was similar to that quoted for 3,5-dichloro-*p*-anisic acid (**4a**) methyl ester (Field et al., 1996). The other esters identified on the basis of GC–MS analysis were those of cinnamic (*m/z* 162, M^+; and *m/z* 131 due to loss of methoxy group), phenylacetic (*m/z* 150, M^+ and *m/z* 91 due to formation of tropylium ion), and pent-4-ynoic (*m/z* 162, M^+).

The neutral fraction upon chromatographic analysis gave two dichloroanisole derivatives, namely the 3,5-dichloro-4-methoxybenzaldehyde (**4**) and 3,5-dichloro-4-methoxybenzyl alcohol (**5**) as well as traces of the free acid (**4a**). Their mass spectra showed peak intensity ratio typical of two chlorine atoms (McLafferty, 1973). The presence of the acid **4a** in the neutral fraction could be a product of air oxidation of the aldehyde (**4**).

Compound **4** had physical properties and spectral data consistent with the molecular formula $C_8H_6O_2Cl_2$ and identical to that reported in the literature (De Jong *et al.,* 1994). Its EI-mass spectrum had following peak intensities: *m/z* 208 $[M+4]^+$(10), 207 $[M+3]^+$(18), 206 $[M+2]^+$ (65), 205 $[M+1]^+$ (75), 204 $[M^+]$ (100) 203 (100), 161 (18), 135 (28), 133 (48), 111 (20), 109 (18), 97 (38), 75 (28), 74 (30), 73 (22), 63 (20), 62 (30) and 61 (21) which is typical to that reported for dichloroanisoles (Field et al., 1996); typical aromatic absorption was also



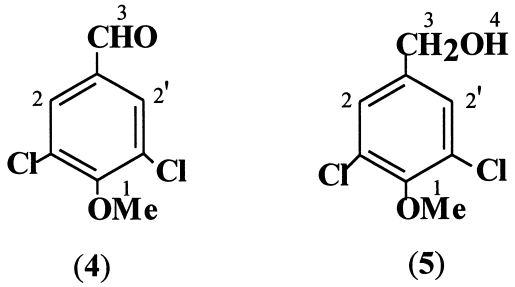


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Fig. 1. Structures of compounds isolated from *Psathyrella scobinacea* culture fluids.

shown by UV (Et_2O) λ_{max} 260 (rel. $E=1$) and 225 (1.5) nm; IR absorptions were present at 1700 (aromatic aldehyde) and 1600 (benzene ring) cm^{-1} ; ^1H NMR ($CDCl_3$, 300 MHz) showed resonances at $\delta=4.0$ (*s*, methoxy group protons, H-1), 7.85 (*s*, two aromatic protons, H-2), and 9.90 (*s*, aldehydic proton, H-3). Upon analysis of fraction (II) by flash chromatography (Et_2O–petrol, 1:4) gave two further fractions (IIa) and (IIb). The less polar fraction (IIa) on purification by preparative-TLC ($CHCl_3$, continuous elution, 2.5 h) gave needles of scobinolide, the isolation and synthesis of which have been described previously (Gadir et al., 1986). Fraction (IIb) upon purification by preparative TLC ($CHCl_3$, continuous elution, 3 h) gave crystals (Et_2O) of **5**, melting point 42°C.

Compound **5** had physical properties identical to that reported in the literature (De Jong et al., 1994) with spectral data consistent with the molecular formula $C_8H_8O_2Cl_2$ and similar to compound **4** above except for the presence of hydroxyl functionality instead of the aldehyde. Its EI mass spectrum had following peak intensities: *m/z* 210 $[M+4]^+$ (10), 208 $[M+2]^+$ (65), 206 $[M^+]$ (100), 171 (94), which is comparable to that reported for the dichloroanisloes (Field et al., 1996). Typical aromatic absorption was shown by UV (Et_2O) λ_{max} 283 (rel. $E=1$), 275 (1) and 206 (6) nm; IR absorptions at 3620, 3500 (hydroxyl group) and 1600 (benzene ring) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) showed resonances at $\delta=2.25$(*s*, D_2O exchange, hydroxyl proton, H-4), 3.81 (*s*, methoxy protons, H-1), 4.50 (s, CH_2O protons, H-3), and 7.20 (s, two aromatic protons, H-2). The presence of the aldehyde (**4**) and the alcohol (**5**) in *P. scobinacea* and in other fungal cultures such as the genus *Bjerkandera* (De Jong et al., 1994) which also contained the acid (**4a**) shows these compounds to have most likely a common biogenesis. The fragmentation pattern as shown in the $[M+4]^+$, $[M+2]^+$ and $[M]^+$ ions and their relative intensities were found to be typically similar to those given for the chlorometabolites of *Bjerkandra* species (Field et al., 1996). Similar chlorinated aromatic aldehydes have been isolated previously from *Lepista diemii* (Thaller and Turner, 1972) and *L. nudi* (Asplund et al., 1996). The antibiotic drosophilin A is a tetrachlorinated phenol isolated from *Psathyrella subatrata* (Field et al., 1997). The acid (**4a**) and other related chloroanisoles, due to facile decarboxylation in alkaline medium followed by *O*-demethylation by soil micro-organisms to form chlorinated phenols, has been implicated to be a possible precursor to the environmental pollutants, the dioxins (Field et al., 1996).

Apart from the two chlorinated anisoles (**4** and **5**) five acetylenic alcohols appear to be present in the neutral fraction of culture fluids of the *P. scobinacea*. These are the C10 triynols (**6**) and (**7**), the C7 diynediol (**8**) and the *cis* and *trans* isomers C7 enynediols (**9**) and (**10**). Fraction III (100 mg) was separated by preparative TLC (Et_2O–petrol, 2:3, continuous elution, 3 h) into two bands A and B. A yielded the polyacetylenic alcohol deca-5,7,9-triyn-1-ol (**6**) (10 mg), as a colourless liquid with a sharp smell.

Compound **6** had a triyne structure consistent with the molecular formula $C_{10}H_{10}O$. Its IR spectrum showed a hydroxyl function (3650 cm^{-1}) with a C–O absorption at 1056 cm^{-1} and a terminal acetylene absorptions at (3320 and 2120) cm^{-1}. The polyacetylenic nature of the alcohol was shown by the presence of another band at 2240 cm^{-1}. Resonances in the ^1H NMR. spectrum at $\delta=1.25$ (*s*, D_2O exchange, H-5) and at $\delta=1.80$ (*s*, H-1) were due to hydroxyl and terminal acetylenic groups, respectively. Signals at $\delta=1.75$ (multiplet of four protons, H-3), at $\delta=2.27$ (*t*, $J=7$ Hz, H-2) and at $\delta=3.70$ (*t*, $J=6$ Hz, H-4) were due to methylene groups. The EI-mass spectrum of the compound gave a molecular ion at *m/z* 146 (20%). The base peak at *m/z* 77 (phenyl group) and the other major peak at *m/z* 91 (50%, tropylium ion) were consistent with the tendency of terminal triynes to cyclise into $C_6H_5^+$ and $C_7H_7^+$. Fragments at *m/z* 132 (loss of CH_2), *m/z* 128 (loss of H_2O) and *m/z* 115 (loss of CH_2OH) are consistent with the proposed structure of a polyacetylenic alcohol which is saturated at the hydroxyl end of the molecule.

Compound **7** (0.85 mg l^{-1} of culture fluid) had a triynol structure consistent with the molecular formula $C_{10}H_{10}O$ similar to **6**. However the compound showed no terminal IR absorptions at 3320 and 2120 cm^{-1}, but bands at 3650 (hydroxyl group), 2238 (C≡C), and 1060 (C–O) cm^{-1}. ^1H NMR signals were comparable with those of the triynol (**6**) except for the absence of a terminal ethynyl proton signal at $\delta=1.80$ and the presence of a singlet at $\delta=1.95$ (H-1) due to a methyl on a triple bond. Signals at $\delta=2.40$ (*t*, $J=7$ Hz, H-2) and $\delta=3.70$ (*t*, $J=6$ Hz, H-4) were due to two different methylene groups; whereas a multiplet at $\delta=1.76$ (H-3) integrated for another two methylene protons. The signal at $\delta=1.40$ (*s*, D_2O exchange, H-5) was ascribed to the hydroxyl moiety. In the mass spectrum the molecular ion at *m/z* 146 (100%) was also the base peak and peaks at *m/z* 131 (10%), *m/z* 128 (32%) and *m/z* 115 (60%) corresponded to loss of–CH_3, H_2O and–CH_2OH, respectively.

Compound **8**, a colourless viscous oil, had a diynediol structure consistent with the molecular formula $C_7H_8O_2$. It was obtained from fraction IV (90 mg) following its separation by preparative TLC (Et_2O–petrol, 1:1, continuous elution, 3 h) into two bands A & B. Band B, R_f 0.32 (Et_2O) gave compound **8** (10 mg), as an oil soluble in $CHCl_3$ but insoluble in CCl_4. The compound had leavorotatory ORD: $[\alpha]^{20}-20.2°$ (589 nm), (*c*, 1.85, EtOH). The EI-mass spectrum gave no observable molecular ion peak but a base peak at *m/z* 80 due to loss of CH_3CH^+OH from $(M+1)^+$ ion. This

fragmentation is characteristic of a glycol cleavage (compare for instance glycol cleavage in the mass spectrum of the fungal antibiotic trichoviridin) (Godtfredsen et al., 1980). A peak at *m/z* 62 (19%) was due to the loss of H_2O and another one at *m/z* 45 (70%) was due to CH_3CH^+OH. A molecular ion, *m/z* 124 (3%), was eventually obtained using the CI-MS technique. UV spectrum of **8** showed a weak absorption, λ_{max} (Et_2O) 252.2(log $\epsilon = 2.38$), typical of a diyne absorption. Strong bands in the IR spectrum at 3600 and 3320 cm^{-1} were due to hydroxy and terminal acetylene groups, respectively. The ^1H NMR spectrum had an AX_3 system due to coupling between the methyl protons H-1 ($\delta = 1.30$, *d*, $J = 6.6$ Hz) and H-2 which is further split (*dq*, $J = 7.0$, 6.6 Hz) by H-4. A singlet at $\delta = 2.20$ was due to the terminal ethynyl proton H-6; whereas two broad singlets (disappeared on D_2O exchange) at $\delta = 2.95$ and 3.25 were assigned to two hydroxyl protons H-3 and H-5.

Two more diols named scobinynediol-II (**9**) and-II (**10**) were detected in minute quantities; their spectral data, especially the 300 MHz, suggested the presence of structures *cis* (**9**) and *trans* (**10**). The mass spectrum of the *cis*-diol (**9**) did not give a molecular ion but the main fragments at *m/z* 81 (95%) and at *m/z* 45 (100%) were ascribed to the diol cleavage with the base peak due to (CH_3CHOH^+) fragment. The *cis*-diol similar to the diynediol (**8**) described above had a negative molecular rotation. The diols were obtained from band B, R_f 0.35 (Et_2O–petrol–EtOAc, 9.5:9.5:1) as a mixture of *cis*/*trans* isomers (10 mg) which was separated further by preparative TLC (Et_2O–petrol–EtOAc, 9.5:9.5:1, continuous elution, 1 h) into the *cis*-(**9**) (5 mg) and the *trans*-(**10**) (2 mg). They had the following spectral data: *cis*-diol had molecular rotations $[\alpha]^{20}$ at $-3.2°$ (578), $-4.3°$ (546), $-10.7°$ (436) and $-17.6°$ (365 nm) (*c*, 1.87, $CHCl_3$). EI–MS (no M^+ ion peak) gave peaks at *m/z* 81 ($M^+ -45$) (95%), and 45 (100%); UV (Et_2O) measurements gave a major band at 223.5 (log $\epsilon = 3.71$) nm; IR ($CHCl_3$) bands were at 3590 (–OH), 3320 (C≡C–H), 3000–2900 (C–H) and 2120 (C≡C–H) cm^{-1}; whereas IR (CS_2) absorptions gave the *cis* double bond band at 720 cm^{-1}; the 300 MHz ^1H NMR ($CDCl_3$) showed a doublet due to methyl group at $\delta = 1.22$ (*d*, $J = 6.2$ Hz, H-1), which is coupled to proton H-2 ($\delta = 3.72$, *dq*, $J = 7.0$, 6.2 Hz), and a broad peak at $\delta = 2.5$ (*br*, 2-OH; disappeared on addition of D_2O, H-3 and H-5) due to two hydroxyl groups. Proton H-4 $\delta = 4.45$, *ddd*, $J = 8.7$, 7.0, 0.8 Hz) showed two ^3J couplings to H-6 (8.7 Hz) and to H-2 (7.0 Hz) and a remote ^4J coupling to H-7 (0.8 Hz). The two protons H-6 $\delta = 5.67$, *ddd*, $J = 10.3$, 8.7, 0.8 Hz) and H-7 ($\delta = 6.00$, *ddd*, $J = 10.3$, 2.5, 0.8 Hz) flank a *cis* double bond ($J = 10.3$ Hz). These two protons, apart from being coupled to H-4, each shows one more coupling; H-6 shows a ^5J long range coupling to the terminal acetylenic proton H-8 ($J = 0.8$ Hz) whereas H-7 shows a ^4J long range coupling to the same proton H-8 ($J = 2.5$ Hz). The *trans*-diol (**10**): IR ($CHCl_3$) 3590 (–OH), 3320 (C≡C–H), 3000–2900 (C–H), 2120 (C≡C–H) and 965 (*trans* CH=CH) cm^{-1}; the 300 MHz ^1H NMR ($CDCl_3$) was similar to that of **9** except for the presence of a *trans* double bond. A doublet at $\delta = 1.22$ (*d*, $J = 6.2$ Hz, H-1) was due to a methyl group, which is coupled to proton H-2 ($\delta = 3.70$, *dq*, $J = 7.1$, 6.2 Hz), and a broad peak at $\delta = 1.70$ (*br*, 2-OH; disappeared on addition of D_2O, H-3 and H-5) was due to two hydroxyl groups. Proton H-4 ($\delta = 3.94$, *ddd*, $J = 7.1$, 5.0, 0.8 Hz) showed two ^3J couplings to H-2 (7.1 Hz) and to H-6 (5.0 Hz) and a remote ^4J coupling to H-7 (0.8 Hz). The two protons H-6 ($\delta = 5.85$, *ddd*, $J = 16.5$, 5.0, 0.8 Hz) and H-7 ($\delta = 6.30$, *ddd*, $J = 16.5$, 2.4, 0.8 Hz) flank a *trans* double bond ($J = 16.5$ Hz). These two protons, apart from being coupled to H-4, each shows one more coupling; H-6 shows a ^5J long range coupling to the terminal acetylenic proton H-8 ($J = 0.8$ Hz) whereas H-7 shows a ^4J long range coupling to the same proton H-8 ($J = 2.4$ Hz). Unequivocal proof of structure and stereochemistry of these three diols via synthesis is underway.

3. Experimental

3.1. General

UV: Unicam SP800A; IR: Unicam SP1000. ^1H NMR: Brucker WH 300, spectra recorded at 300 MHz with $CDCl_3$ as solvent and TMS as int. standard; MS: (EIMS and CIMS) were recorded on a VG Micromass 16F, VG ZAB-IF and Varian MAT CH7 mass spectrometers (70 eV) in the temperature range 25–200°C, using direct insertion probes. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. CC: Silica gel (H.B.L. M60); TLC: Precoated thin layer plates (Merck, silica gel $HF_{254+366}$) and prep. TLC (Merck, silica gel $PF_{254+366}$, 1 mm layers). M.ps:corr., Kofler hot-stage apparatus. Petrol (light petroleum): bp 30–40°C.

3.2. Fractionation of P. scobinacea culture fluids

The fungus growth conditions were as described previously (Gadir et al., 1986). The combined culture fluids were saturated (NaCl) and extracted continuously with Et_2O for 48 h. The ether extract from 50 flasks (35 l culture fluid) was concentrated to 200 ml, separated into neutral and acid fractions (saturated $NaHCO_3$ solution) and the acid fraction was esterified ($MeOH–H_2SO_4$, 24:1, 48 h).

3.3. Isolation of the neutral and acid fractions

The methyl esters (500 mg) of the acid fraction were separated by column chromatography (silica gel, 100 g)

by stepwise elution (Et_2O–petrol, 1:9 to MeOH–Et_2O, 1:10) and 10 fractions (I–X) were collected. Fractions (III–X) showed no typical UV and IR absorptions and were discarded. Fractions (I–II) (100 mg) were further separated by prep. TLC (Et_2O–petrol, 1:19) and by flash chromatography (Et_2O–petrol, 1:19) into bands A, B and C in order of increasing polarity. The neutral fraction (400 mg) was similarly separated by column chromatography (silica gel, 100 g) by stepwise elution (Et_2O–petrol, 1:9 to MeOH–Et_2O, 1: 10) into four major fractions (I–IX).

3.4. Octa-2,4,6-triynoic acid (*1*)

Methyl ester from band A as plates (petrol) (0.3 mg l^{-1} of culture fluid); mp 54–57°C, lit. 53–56°C (Jones et al., 1957); UV $\lambda_{max}^{Et_2O}$ nm (rel. *E*) 327 (3.8), 306 (6.4), 287 (5), 271 (2.8), 257 (1.5), 244 (1), and 225 (67.2); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2240 (C≡C stretching), 1735 (ester carbonyl); ^1H NMR (300 MHz, $CDCl_3$): Table 1; EIMS *m/z* (rel. int.) 146 (M^+, 88), 115 (100), 103 (12), 87 (44), and 61 (18).

3.5. Dec-trans-2-ene-4,6,8-triynoic acid (*2*)

From band B the methyl ester of dehydromarticaria acid (**2**) was isolated (0.6 mg l^{-1} of culture fluid), mp 104–106°C, lit. 105–106°C (Gardner et al., 1960); UV $\lambda_{max}^{Et_2O}$ nm (rel. *E*) 344 (2.5), 320 (2.7), 301 (2.4), 283 (1.94), 268 (1.0), 255 (3.3), and 243 (3.1); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2239 (C≡C stretching), 1725 (ester carbonyl) and 950 (trans CH=CH); ^1H NMR (300 MHz, $CDCl_3$): Table 1; EIMS *m/z* (rel. int.) 172 [M^+](100), 144 (20), 141 (23) and 113 (14).

3.6. Dec-cis-2-ene-4,6,8-triynoic acid (*3*)

The material from band C was crystallized (petrol) and gave methyl dec-*cis*-2-ene-4,6,8-triynoate (**3**) (5 mg) mp113–114°C, lit. 112–115°C (Bell et al., 1956); EIMS *m/z* (rel. int.) 172 [M^+] (100), 144 (22), 141 (26) and 113 (18); UV $\lambda_{max}^{Et_2O}$ nm (rel. *E*) 345 (2.0), 323 (2.2), 303 (1.8), 285 (1.0), 270 (0.8), 255 (2.6), and 243 (2.4).

3.7. 3,5-Dichloro-4-methoxybenzaldehyde (*4*)

Upon analysis by flash chromatography (Et_2O–petrol, 1:9) fraction I (120 mg) gave crystals (petrol) of 3,5-dichloro-4-methoxybenzaldehyde (**4**) (60 mg) mp 60°C ; UV $\lambda_{max}^{Et_2O}$ nm (rel. *E*) 260 (1.0) and 225 (1.5); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700 (Ar–CHO) and 1600 (Ar–H) cm^{-1} ; ^1H NMR (300 MHz, $CDCl_3$): Table 1; EIMS *m/z* (rel. int.) 208 $[M+4]^+$ (10), 207 $[M+3]^+$ (18), 206 $[M+2]^+$ (65), 205 $[M+1]^+$ (75), 204 [M^+] (100), 203 (100), 161 (18), 160 (8), 135 (28), 133 (48), 111 (20), 109 (18), 97 (38), 75 (28), 74 (30), 73 (22), 63 (20), 62 (30) and 61 (21).

3.8. 3,5-Dichloro-4-methoxybenzyl alcohol (*5*)

Crystals (Et_2O) (30 mg) mp 42°C; UV $\lambda_{max}^{Et_2O}$ nm (rel. *E*) 283 (1.0), 275 (1.0) and 206 (6.0); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620, 3500 (–OH), 1600 (Ar–H), 1485, 1430, 1270, 1200, 1085, 1000 and 860 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): Table 1; EIMS *m/z* (rel. int.) 210 $[M+4]^+$ (10), 208 $[M+2]^+$ (65), 206 [M^+] (100), 171 (94), 143 (46), 142 (44), 141 (56), 128 (25), 111 (25), 108 (30), 101 (20), 99 (63), 79 (22), 77 (37), 75 (35), 74 (17) and 73 (25).

3.9. Deca-5,7,9-triynol (*6*)

Colourless liquid (10 mg); UV $\lambda_{max}^{Et_2O}$ nm (rel. *E*) 305 (0.1), 284 (0.2), 266 (0.2), 252 (0.2), 239 (0.1) and 212 (152); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3650 (–OH), 3320 (C≡C–H), 3000–2900(C–H), 2240 (C≡C), 2120 (C≡C–H), 1440 and 1056 (C–O) cm^{-1}; ^1H NMR (300 MHz, CCl_4): Table 1; EIMS *m/z* (rel. int.) 146 [M^+] (20), 132 (40), 128 (10), 115 (20), 103 (75), 102 (60), 91 (50), 79 (70), 78 (75), 77 (100), 65 (55) and 63 (60).

3.10. Deca-4,6,8-triynol (*7*)

From (III) B crystals (Et_2O–petrol, 1:2, 0°) (30 mg), mp 72–73°C, lit. 72°C (Bently et al., 1969); UV $\lambda_{max}^{Et_2O}$ nm (log ϵ) 305 (−1), 284 (−0.7), 266 (−0.7), 252 (−0.7), 239 (−1) and 213 (5.16); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3650 (–OH), 3000–2900 (C–H), 2238(C≡C), 1430, 1380, 1355 and 1060 (C–O)

Table 1
^1H NMR spectral data of *P. scobinacea* compounds (**1**,**2**,**4**–**10**) in $CDCl_3$ as solvent[a]

H	1	2	4	5	6[b]	7[b]	8	9	10
1	2.00 *s*	2.00 *s*	4.00 *s*	3.81 *s*	1.80 *s*	1.95 *s*	1.30 *d* (6.6)	1.22 *d* (6.2)	1.22 *d* (6.2)
2	3.80 *s*	6.80 *d* (18)	7.85 *s*	7.20 *s*	2.27 *t* (7)	2.40 *t* (7)	3.82 *dq* (7, 6.6)	3.72 *dq* (7, 6.2)	3.70 *dq* (7.1, 6.2)
3		6.35 *d* (18)	9.90 *s*	4.50 *s*	1.75 *m*	1.76 *m*	2.95 *br*	2.50 *br*	1.70 *br*
4		3.80 *s*		2.25 *s*	3.70 *t* (6)	3.70 *t* (6)	4.15 *d* (7)	4.45 *ddd* (8.7, 7, 0.8)	3.94 *ddd* (7.1, 5.0, 0.8)
5					1.25 *s*	1.40 *s*	3.25 *br*	2.50 *br*	1.70 *br*
6							2.20 *s*	5.67 *ddd* (10.3, 8.7, 0.8)	5.85 *ddd* (16.5, 5.0, 0.8)
7								6.00 *ddd* (10.3, 2.5, 0.8)	6.30 *ddd* (16.5, 2.4, 0.8)
8								3.18 *dd* (2.5, 0.8)	2.93 *dd* (2.4, 0.8)

[a] 300 MHz, δ values from internal TMS, *J* Hz in parentheses.
[b] δ(CCl_4).

cm^{-1}; ^1H NMR (300 MHz, CCl_4): Table 1; EIMS: *m/z* (%, rel. int.) 146 (M^+, 100), 131 (10), 128 (32), 115 (60), 102 (90), 101 (39), 89 (49) and 87 (25).

3.11. Hepta-4,6-diyne-2,3-diol (**8**)

Viscous liquid (10 mg), b.p. 47–50°C (Block) at 15 mm Hg; $[\alpha]^{20}$ −20.2° (589), −21.1° (578), −24.2° (546), −43.6° (436) and −72.7° (365 nm) (*c*, 1.85, EtOH); UV $\lambda_{max}^{Et_2O}$ nm (log ϵ) 252.5 (2.4), 239 (2.6), 227.5 (2.6) and 216 (2.6); IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3600 (–OH), 3320 (C≡C–H), 3000–2900 (C–H), 2120 (C≡C–H), 1395, 1380, 1130 and 1020 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): Table 1; EIMS *m/z* (%, rel. int.) 80 [$M^+ + 1$]−(45), (100), 62 (19), 52 (39), 51 (25), and 45 (70). CIMS *m/z* (%, rel. int.) 125 ($M^+ + 1$) (2), 124 (M^+) (3), 107 (7) and 79 (100).

3.12. Hept-4-cis-en,6-yne-2,3-diol (**9**)

As a mixture of *cis*/*trans* isomers (10 mg) separated by prep. TLC (Et_2O–petrol–EtOAc, 9.5:9.5:1, continuous elution, 1 h) into the *cis*-(**9**) (5 mg) and the *trans*-(**10**) (2 mg). Spectral data: *cis*-diol $[\alpha]^{20}$ −3.2° (578), −4.3° (546), −10.7° (436) and −17.6° (365 nm) (*c,* 1.87, $CHCl_3$); UV $\lambda_{max}^{Et_2O}$ nm (log ϵ) 223.5 (3.7); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590 (–OH), 3320 (C≡C–H), 3000–2900 (C–H), 2120 (C≡C–H), 1390 and 1380; IR $\nu_{max}^{CS_2}$ cm^{-1}: 3320 (C≡C–H) and 720 (*cis*-CH=CH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): Table 1 ; EIMS *m/z* (%, rel. int.) 107 (40), 82 (50), 81 (M^+ −45) (95), 63 (10), 54 (50), 53 (40) and 45 (100).

3.13. Hept-4-trans-en,6-yne-2,3-diol (**10**)

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590 (–OH), 3320 (C≡C–H), 3000–2900 (C–H), 2120 (C≡C–H), 1390, 1380, 1130, 1020, and 965 (*trans* CH=CH); and ^1H NMR (300 MHz, $CDCl_3$): Table 1.

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