Phytoactive Eremophilanes Produced by the Weed Pathogen *Drechslera gigantea*


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Phytoactive substances were present in a culture broth of *Drechslera gigantea*, a pathogenic fungus of several grasses. Twelve eremophilane sesquiterpenes (1, 2, 3, 4, 6, 7, 10, 11, 12, 13, 14, and 15) were isolated and structurally characterized by a combination of single-crystal X-ray diffraction and spectroscopic analyses. Most of these sesquiterpenoids were phytotoxic; however, compounds 1 and 3 caused chlorophyll retention, an activity previously associated with phytohormones.

The plant pathogenic fungus, *Drechslera gigantea*, infects several grasses including such common weeds as crabgrass (*Digitaria* spp.), quackgrass (*Agropyron repens*), and Bermuda grass (*Cynodon dactylon*). Dark brown lesions with green borders characterize the fungal infection, and these symptoms typify the effects of fungal-produced phytoactive metabolites. As part of a continuing program to explore fungal weed pathogens as sources of novel phytoactive compounds, we investigated culture broths of *D. gigantea*. The fungus was cultured on a semi-synthetic medium, and two- to three-week old culture broths were extracted. Phytoactivity was used to guide the fractionation of the extracts to yield a number of eremophilane sesquiterpenes, and these are the subject of this report.

Several of the compounds produced by our culture of *D. gigantea* have been discussed in previous reports. We recently reported both the structural elucidation of gigantene (1) by single-crystal X-ray diffraction and its phytotoxic-like activity. Gigantene (1) is related to phaseolinone (2), a phytotoxin which has been previously isolated from *Macrophomina phaseoli*, while petasol (3) has been found in the higher plant *Petasites hybridus*, and phomenone (7) (7) was from *Phoma exigua*. The rest of the phytoactive eremophilanes produced by *D. gigantea* are new.

**Gigantene (1) and phaseolinone (2)**

While gigantene (1) was ultimately characterized by X-ray diffraction, most of the structure was clear from spectroscopic measurements. The NMR spectrum of known sesquiterpene phaseolinone (2) was clearly related to that of 1. The major difference being in the 13C-NMR spectrum of 2, the primary alcohol carbon (C-13) resonating at δ 62.1 (t) being replaced in 1 by a methyl carbon at δ 18.8 (q). The proton resonances from the other methyl groups (δ 1.27 and 1.10) and epoxide signals (δ 2.84 and 2.64 for H-12α and H, and δ 3.65 for H-6) of 1 were closely comparable to those of 2. Another important comparative compound was sporoigen AO-1 (9) which has been characterized by X-ray diffraction. The coupling constants of the protons at C-1, C-2, C-3, and C-4 in 9 allowed a full characterization of 1 by comparison. Since the stereochemistry at C-11 has been suggested to be R in gigantene (1), it is reasonable that the stereochemistry at C-11 in phaseolinone (2), which was previously undetermined, would also be R.

**Sesquiterpenes 3 and 4**

Petasol (3), a known eremophilane from higher plants, was used to elucidate the structure of compound 4. The formula of 4, C15H22O3, differed from that of 3 by one
oxygen, and this, in conjunction with very similar spectra for 3 and 4, suggested that 4 was a hydroxypetasol derivative. The allylic methyl group (C-13) of petasol (3), which occurred at δ 1.74 in the 1H-NMR spectrum of 3, was absent from the NMR spectrum of 4. In place of the missing methyl signal, an AB quartet was present at 6 4.18 and 4.12 (J = 13.0 Hz). Since no other differences between the NMR spectra of 3 and 4 were apparent, compound 4 must have been 13-hydroxypetasol. The coupling of H-7 with both H-6ax and H-6ex (dd, J = 4.6 and 14.4 Hz) can best be explained on the basis of the stereochemistry shown, i.e., a structure with the same stereochemistry as that of petasol (3). The structure of 4 was further confirmed by oxidizing with MnO₂ in CH₃Cl₂ to give α,β-unsaturated aldehyde 5.

Sesquiterpenes 6 and 7

Analysis of compound 6 by HREIMS established a molecular formula of C₁₅H₂₄O₃. The 1H-NMR spectrum had a new peak at δ 4.45 (H-1) that was not present in any of the compounds already discussed. Irradiation of this new peak led to a simplification of the signals at δ 2.30 (fromddd to dd, H-2ex) and 1.60 (complex due to overlapping with other methylene protons, H-2ax). In the 13C-NMR spectrum of 6, two signals corresponding to alcohol carbons (δ 73.7 and 67.3) were observed. A DEPT experiment showed the presence of three methylenes, one being the exo-olefin group. Thus, one of the three methylenes on the eremophilane ring system, C-1, C-2, or C-6, was a secondary alcohol.

To determine the site of oxygenation, a series 1H-1H decoupling experiments was carried out. Correlation of the results from these decoupling experiments led to the conclusion that compound 6 was 1-hydroxypetasol. Since no coupling was observed for the allylic proton (H-1) and the olefinic proton (H-9), we deduced that the alcohol had the β orientation, i.e., an R configuration. The large coupling that was observed between H-3 and H-4 (J = 14.0 Hz) suggested that these two protons were diastereotopic, as in the previously studied case. The multiplicities of the carbons were assigned from DEPT spectral data and are in agreement with proposed structure 6. Oxidation of the C-1 carbon of an eremophilane ring system has been observed in PR-toxin, eremofortins A—C, capsidiol, and 1-hydroxydebeneyol.

The spectral data of compound 7 were identical to those of phenomeone, which was previously isolated from phoma exigua and structurally characterized by X-ray diffraction methods. MnO₂ oxidation of 7 afforded a compound whose spectral data were consistent with aldehyde 8.

Sesquiterpene 10

Phomenone (7) was the most useful comparative compound for the structural analysis of 10. The 1H-NMR spectrum of 10 was similar to that of phenomeone, with the exception of resonances from the isopropenyl residue. The vinylic methyl group in phenomeone moved to a higher field (δ 1.46), and the signals for the exo-olefin protons disappeared, while the signals corresponding to the epoxide and primary alcohol remained. The most likely structure of compound 10 was 11-hydroxyphenomeone, and this formulation was consistent with the molecular formula, C₁₅H₂₂O₂, given by HREIMS. However, the stereochemistry of C-11 remains unsettled. Hydroxylation at C-11 has been previously observed in other eremophilanes. As a related observation, although 13-deoxyphenomenone (=sporogen AO-1) 9 has been isolated from Hanfordia pulvinata and Aspergillus oryzae, it was not found in D. gigantea.

Sesquiterpene 11

When the spectral data of compound 11 were compared to those of compound 10, they were very similar except for the signals representing the isopropenyl side chain. The methyl group resonance for C-13 had disappeared, and a second AB quartet at δ 4.12 and 3.94 (J = 11.6 Hz, H-13a and b) had appeared. This additional AB quartet taken in conjunction with its large coupling constant implies that there were two primary alcohol groups in 11. A detailed 1H-NMR spectral analysis, including exhaustive one-dimensional decoupling experiments, provided the most reasonable structural formulation as 11. However, the relative stereochemistry at C-6 could not be determined.

Sesquiterpene 12

Compound 12 had the molecular formula C₁₅H₂₂O₂, two mass units less than petasol (3). Its 1H-NMR spectrum showed a much simpler pattern at δ 1.5 to 2.5, while an additional olefin proton signals appeared at δ 6.89. The 1H-NMR and MS data suggest that 12 was 6-dehydropetasol.

Sesquiterpene 13

Compound 13, a minor component, was isolated only from the butanol extracts. Its characterization was accomplished by comparing its spectral data with 6-dehydropetasol (12). Compound 13 differed from 6-dehydropetasol (12) only on the isopropenyl side chain, the rest of the structural features being unchanged. Since the exomethylene protons at C-12 of 12 had disappeared, and a primary alcohol signal at δ 3.81 and 3.55, typical of a primary alcohol system, had appeared, compound 13 was determined to be 6-dehydro-11,12-dihydroxypetasol. This analysis was supported by the molecular formula (C₁₅H₂₂O₄) obtained from high-resolution EIMS. The limited quantity of this material, however, did not allow the complete assignment of the relative stereochemistry at C-11. The relative
stereochemistry of the A-ring seemed unchanged, since the coupling constants were comparable to those structures already discussed. Unsaturation at C-6 of an eremophilane system has also been seen in petasinin, a metabolite from the plant *Petasites japonicus* Maxim.\(^\text{33}\)

**Sesquiterpene 14**

The \(^1\)H-NMR spectrum of compound 14 showed the disappearance of the exo-olefin functionality of petasol (3). It had been replaced by two doublet signals at \(\delta 2.85\) and 2.74 (\(J = 4.6\) Hz). The molecular formula \(\text{C}_{15}\text{H}_{23}\text{O}_{5}\), established by HREIMS, is consistent with that of 11,12-epoxyterpsol (14). The coupling constants between the H-6 protons and H-7 (\(J = 4.6\) and 14.4 Hz) suggest that H-7 was *axial*, and therefore, the stereochemistry at C-7 was the same as that of petasol (3). The stereochemistry at C-11, however, could not be defined from our data.

**Sesquiterpene 15**

The lower-field region of compound 15's \(^1\)H-NMR spectrum was the same as that of petasol (3) while the higher-field region showed some differences. HREIMS analysis gave a molecular formula of \(\text{C}_{15}\text{H}_{22}\text{O}_{3}\). Homonuclear decoupling experiments allowed us to assign the two doublets at \(\delta 2.36\) (H-6ex, overlapped with H-1ax signals) and 1.78 (H-6ax), typical of geminally coupled protons. Based on this information, compound 15 was assigned as a C-7 hydroxyterpsol. The stereochemistry at C-7 was tentatively assigned to be *S* by virtue of its negative optical rotation (\(\delta 220 = -47^\circ\)).

**Biological activity**

Eremophilanes are well-known secondary metabolites of both fungi and higher plants.\(^\text{44}\) In the case of *D. gigantea*, the new eremophilane sesquiterpenes produced are generally oxidized variants of previously known ones. We do not have a clear understanding of how the structural variations affect biological activity, the biological effects depending not only on the structure of the eremophilane but also on the plant family. For example, eremophilanes 1, 2, 3, and 7, when placed on a leaf puncture wound on monocots (10–20 \(\mu\)mol), produced green islands (>20 mm²), while the other compounds mentioned in the report either caused no effect or produced small green islands (<10 mm²). With the exception of 6, 10, and 11, all of the eremophilanes produced necrotic lesions on dicots at 10–20 \(\mu\)mol. Since these biological effects were caused by this array of eremophilanes, it would be difficult to imagine that they do not have some role in the development of one or more of the symptoms associated with *D. gigantea* infections in grass plants.

**Experimental**

NMR spectra were recorded on Bruker WM-250 and JEOL GX-400 FTNMR spectrometers. Chemical shifts were taken in \(\delta\) units relative to \(\text{Me}_2\text{Si} (= 0 ppm)\) with \(\text{CDCl}_3\) as the solvent. UV spectra were obtained from a Hitachi 220A spectrophotometer, and mass spectra were recorded on a VG Analytical 70S or Hitachi M-80 instrument. Optical activities were determined with a Perkin-Elmer Model 241MC polarimeter. Column chromatography adsorbents included Kiesel Gel 60 (230–

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(1H, s, H-12), 4.45 (1H, dd, J = 3.0, 3.0 Hz, H-1), 4.03 (1H, ddd, J = 4.7, 14.0, 14.0 Hz, H-3), 3.20 (1H, dd, J = 7.4, 13.5 Hz, H-7), 2.30 (1H, ddd, J = 3.0, 4.4, 14.0 Hz, H-2eq), 2.10 (1H, d, d, J = 4.7, 14.0 Hz, H-1eq), 1.90 (1H, dd, J = 13.5, 14.0 Hz, H-6ax), 1.60 (2H, m, H-2ax and H-4), 1.72 (3H, s, H-13), 1.35 (3H, s, H-14), 1.09 (3H, d, J = 6.7 Hz, H-15); 1C-NMR δ: 165.0 (s, C-10), 143.1 (s, C-11), 126.8 (dd, C-5, C-9), 114.6 (t, C-12), 73.7 (d, C-11), 67.3 (d, C-3), 50.9 (dd, C-7), 50.1 (d, C-4), 43.8 (t, C-6), 41.8 (t, C-2), 39.4 (s, C-5), 20.2 (q, C-15), 19.7 (q, C-13), 10.7 (q, C-14).

**Sesquiperpe (10).** 13C NMR (500 MHz, CDCl3): δ 145.2 (C-10); δ 133.1 (C-7); δ 126.6 (C-11); δ 124.9 (C-12); δ 118.7 (C-5, C-9); δ 114.5 (C-13); δ 73.1 (C-11); δ 70.2 (C-3); δ 51.1 (C-7); δ 43.8 (C-6), 41.8 (t, C-2), 39.4 (s, C-5), 20.2 (q, C-15), 19.7 (q, C-13), 10.7 (q, C-14).

**Sesquipeperone (15).** 13C NMR (500 MHz, CDCl3): δ 151.4 (C-10); δ 143.1 (C-7); δ 127.7 (C-11); δ 124.9 (C-12); δ 118.7 (C-5, C-9); δ 114.5 (C-13); δ 73.1 (C-11); δ 70.2 (C-3); δ 51.1 (C-7); δ 43.8 (C-6), 41.8 (t, C-2), 39.4 (s, C-5), 20.2 (q, C-15), 19.7 (q, C-13), 10.7 (q, C-14).

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**References**