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CYCLODEBNEYOL, A FUNGITOXIC SESQUITERPENE FROM TNV INFECTED *NICOTIANA DEBNEYI*

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Key Word Index—*Nicotiana debneyi*; Solanaceae; sesquiterpene; phytoalexin; cyclodebneyol; tobacco necrosis virus; fungitoxicity.

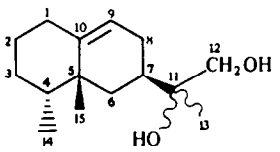
Abstract—A fungitoxic sesquiterpene alcohol, cyclodebneyol, has been isolated from tobacco necrosis virus inoculated leaves of *Nicotiana debneyi*. A structure is proposed from a comparison of its ^{13}C and ^{1}H NMR spectra with those of the closely related sesquiterpene, debneyol.

INTRODUCTION

We have recently [1] isolated and characterized a new antifungal eremophilane-type sesquiterpene, debneyol (**1**), from tobacco necrosis virus (TNV) infected *Nicotiana debneyi*. A structurally identical diol was independently characterized and reported [2] as a phytoalexin from callus tissue of *Nicotiana tabacum*. In this latter paper the resonances of the ^{13}C NMR spectrum of the diol were firmly assigned by comparison with known sesquiterpenes and using DEPT experiments. These data, together with experiments involving the decoupling of the ^{1}H NMR spectrum, have now enabled us to assign the structure of a second antifungal compound from *Nicotiana debneyi* which we isolated previously [1].

RESULTS AND DISCUSSION

The molecular weight of 236, determined from the EI mass spectrum [1], was found to be two mass units lower than that of debneyol and corresponded to a molecular formula of $C_{15}H_{24}O_2$. It indicated the presence of an additional double bond or another ring.



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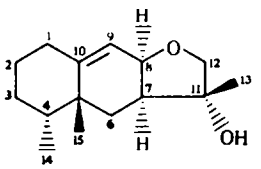

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The ^{13}C NMR spectrum of the unknown showed many common features with that of debneyol (Table 1), particularly at the lower chemical shift end of the spectrum. Thus the signals assigned to carbon atoms 1–5 of ring A as well as those of the C-14 and C-15 methyls occurred at very similar chemical shifts. There was no evidence for any additional olefinic unsaturation and therefore the loss of two mass units relative to debneyol was most likely to be accommodated by the presence of another ring. An important difference from the spectrum of debneyol was the presence of an extra signal in the C–O region (65–85 ppm). This appeared to replace the C-8 methylenic resonance at δ26.4 and in combination with the shifts experienced by C-7, C-11 and C-12 suggested the presence of a tetrahydrofuran ring, as in **2**.

Table 1. ^{13}C NMR spectral data of **1** and **2** (ppm in $CDCl_3$)

Carbon	1*	2†
1	32.0	32.4
2	22.4	23.2
3	30.3	30.4
4	41.6	41.9
5	38.7	39.1
6	38.5	36.8
7	39.3	47.4
8	26.4	74.5
9	120.1	118.2
10	141.6	148.2
11	74.7	81.9
12	68.6	78.0
13	20.1	20.3
14	17.7	17.5
15	30.3	30.1

*Data from refs [1] and [2].
†Assignments were confirmed by a DEPT experiment.

Proof of this structure was obtained from the ^{1}H NMR spectrum and from selective decoupling and nuclear Overhauser enhancement experiments. The protons of the CH_2O group (doublets at δ3.70 and 3.89) were both downfield of the corresponding protons in debneyol and the greater difference between the two CH_2O protons argued for a more rigid structure, a contention supported by the reduced geminal coupling constant (9.5 Hz compared with 10.9 Hz in debneyol [1]). Of the three methyl signals, at δ0.93 (*d*, J = 7.1 Hz), 1.22 (*s*) and 1.32 (*s*), the last one was assigned to the side-chain methyl (C-13), again appreciably downfield of the corresponding signal (δ1.10) in debneyol [1]. These changes in the side-chain signals relative to debneyol are strong supporting evidence for the presence of the tetrahydrofuran ring.

As compared to debneyol [1] the olefinic proton was further deshielded at δ5.75 and appeared as a doublet of doublets (J_1 = 5.6, J_2 = 1.2 Hz), having lost the coupling of 1.7 Hz assigned to an H-9/H-8 coupling in debneyol. This indicated replacement of one of the protons at C-8 by a substituent. Support for this assignment was provided by the appearance of a new one-proton signal for H-8 at δ4.58 (*dd*, J_1 = 5.6 Hz, J_2 = 4.5 Hz), indicating the presence of a methine proton adjacent to oxygen. The couplings of this signal to H-9 and H-7 considered in relation to the likely dihedral angles [3] suggested that the most likely structure has the tetrahydrofuran ring *cis* fused, as in **2**. Confirmation was sought from selective decoupling experiments.

Irradiation of the olefinic proton (H-9) at δ5.75 caused the signal at δ4.58 to collapse to a doublet, confirming that this signal is due to H-8. At the same time, the complex signal at δ2.35 lost a coupling of 1.2 Hz, interpreted as the removal of an allylic coupling to H-9; the remaining couplings shown by this signal (13.1, 13.1 and 5.0 Hz) proved that it was due to the axial proton at C-1, the couplings being to H-1 equatorial, to H-2 axial and to H-2 equatorial respectively.

The other coupling shown by the H-8 methine signal at δ4.58 (4.5 Hz) could have been due to either H-8e/H-7a or to H-8a/H-7e coupling, depending on the precise stereochemistry at C-7 and C-8. Irradiation at δ4.58 caused the signal at δ2.01, now clearly assignable to C-7, to collapse to a doublet of doublets (J_1 = 13.7, J_2 = 4.8 Hz), arising from coupling to the two protons at C-6. The size of the former coupling clearly indicates that it is an axial–axial coupling (to H-6 axial) and therefore that H-7 must also be axial and hence H-8 must be equatorial. This leads to the relative stereochemistry shown in **2** as the only plausible answer, with the stereochemistry at C-11 undefined.

To ascertain the stereochemistry at C-11 relative to that of the rest of the molecule, a nuclear Overhauser enhancement (NOE) difference spectrum was obtained. Irradiation of the C-13 methyl group (δ1.32) caused a large enhancement of the signal at δ3.89 and a much smaller one of the signal at δ3.70; these signals are due to the protons at C-12 but the results do not, on their own, define the relative stereochemistry at C-11. More interestingly, however, the enhancement of the signal due to H-7 (δ2.01) was relatively small and of the same order as that of the signal due to the more distant of the C-12 methylene protons. This requires that the methyl group at C-11 should be approximately *gauche* to H-7, as shown in **2**. In the C-11 epimeric form, the methyl group would be much closer to H-7 than to either of the C-12 protons and would therefore be expected to give a large NOE on H-7 in accordance with the general distance dependence on the NOE [4]. Other signals showing appreciable NOE under these conditions were those at δ1.11 and 1.49, corresponding to H-6a and H-6e respectively. This confirms the assignments arrived at earlier on the basis of the ^{1}H NMR spectrum and selective decoupling experiments.

Taken together, the evidence from ^{1}H and ^{13}C NMR spectra allows structure **2** to be put forward for the new compound from *Nicotiana debneyi*. Since the absolute configurations are not known, the structures shown for **1** and **2** do not imply correct absolute stereochemistry. In view of its relationship with debneyol (**1**), the name cyclodebneyol is proposed for **2**. As expected cyclodebneyol did not form an acetate under normal conditions. In the TLC-*Cladosporium cucumerinum* bioassay [5], cyclodebneyol had antifungal activity comparable with debneyol. One μg samples of each compound gave detectable inhibition zones whereas 10 μg produced extensive areas of inhibition.

Cyclodebneyol is an addition to the small number of known *trans*-dimethyleremophilanes [1]. In view of its structure it is likely to be biosynthesized from debneyol by cyclization of the primary alcohol group onto the C-8 allylic carbon.

EXPERIMENTAL

The isolation from TNV-infected *Nicotiana debneyi*, the purification by CC and TLC, and the EIMS of cyclodebneyol (**2**) have already been described [1]. Its purity was further checked by GLC (10 m × 0.25 mm OV-1 bonded capillary column, 60–290° at 5°/min, 10 psi helium). Under these conditions it had an R_t of 15.3 min compared with 16.7 min for debneyol. ^{1}H NMR (200 MHz, $CDCl_3$): δ0.93 (3H, *d*, J = 7.1 Hz, H-14), 1.10 (1H, *t*, J = 14.0 Hz, H-6_{ax}), 1.22 (3H, *s*, H-15), 1.32 (3H, *s*, H-13), 2.01 (1H, *ddd*, J = 13.7, 4.8, 4.5 Hz, H-7), 2.36 (1H, *dddd*, J = 13.1, 13.1, 5.0, 1.2 Hz, H-1_{ax}), 3.70 (1H, *d*, J = 9.5 Hz, H-12), 3.89 (1H, *d*, J = 9.5 Hz, H-12), 4.58 (1H, *dd*, J = 5.6, 4.5 Hz, H-8) and 5.75 (1H, *dd*, J = 5.6, 1.2 Hz, H-9). ^{13}C NMR (22.5 MHz, $CDCl_3$): see Table 1.

Attempted acetylation of cyclodebneyol (excess Ac_2O–pyridine, room temp., 2 weeks) gave only starting material. Details of the *Cladosporium cucumerinum*-TLC bioassay were as previously given [5].

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