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# SESQUITERPENOIDS RELATED TO THE PHYTOALEXIN DEBNEYOL FROM ELICITED CELL SUSPENSION CULTURES OF *NICOTIANA TABACUM*

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**Key Word Index**—*Nicotiana tabacum*, Solanaceae; tobacco, cell suspension culture, phytoalexin, sesquiterpenoid, eremophilane, 7-*epi*-debneyol, 1-hydroxydebneyol; 8-hydroxydebneyol; debneyol, capsidiol; $^1$H NMR

**Abstract**—Three new sesquiterpenoids, 7-*epi*-debneyol, 1-hydroxydebneyol and 8-hydroxydebneyol have been isolated, along with the phytoalexins capsidiol and debneyol, from cellulase-treated cell suspension cultures of *Nicotiana tabacum*. The identity of the new compounds was confirmed by $^1$H NMR analysis of a series of chemical derivatives and by chemical means It is proposed that the hydrocarbon 4-*epi*-eremophila-9,11-diene is a common intermediate in the biosynthesis of all the named compounds and further that 8-hydroxydebneyol is the direct precursor of the phytoalexin cyclodebneyol

## INTRODUCTION

The sesquiterpenoid phytoalexin capsidiol (**1**) was first isolated from *Capsicum annuum* by Stoessl *et al* in 1972 [1] and later from both *Datura stramonium* [2] and *Nicotiana tabacum* [3], detailed investigations have enabled a complete stereochemical analysis of the compound [4]. Debneyol (**2a**) was first isolated from *N. tabacum* by Rowell *et al* in 1979 [5] but has only recently been examined in detail [6, 7] along with the closely related compound cyclodebneyol (**3**) [8].

In our studies on the biosynthesis and metabolism of these compounds in elicitor-treated cell suspension cultures of tobacco, we have used large batch cultures as a convenient source of 10–50 mg amounts of debneyol. We required this compound for use (i) as carrier material for the isolation and characterization (by derivatisation) of radiolabelled-debneyol biosynthesized (along with capsidiol) from either [1-$^{14}$C]isopentenyl pyrophosphate or [1-$^3$H]farnesyl pyrophosphate in cell-free systems obtained from elicited tobacco cells [9] and (ii) as a starting material for the synthesis of radiolabelled putative intermediates of debneyol and capsidiol for testing in the tobacco cell-free system In the course of characterizing the debneyol, obtained from the cellulase-elicited cultures, by $^1$H NMR it became apparent that a small amount of a minor epimer was present It also became clear that the capsidiol sample obtained from the cultures contained two other sesquiterpenoids in small amounts.

In this paper we report on the isolation and characterization of 7-*epi*-debneyol (**2b**), 1-hydroxydebneyol (**4**) and 8-hydroxydebneyol (**5**) by $^1$H NMR and discuss the possible biosynthetic relationships between all of the named compounds

## RESULTS

Chemical shifts for debneyol (**2a**) and the minor epimer (**2b**) are given in Table 1. These data were taken from the $^1$H NMR spectrum of the initial mixture of epimers (10:1) and confirmed by examination of a sample enriched to about 1:1 by TLC. Data for **2a** are in agreement with those reported previously [6, 7], although we find the couplings for H-9 to be *ca* 6.4, 1.8 and 1 8 Hz. These are in keeping with the expected eremophilene conformation **6** for a 7$\beta$ form in which the cyclohexane ring exists in a fixed chair form and the cyclohexene ring in an envelope form. Torsion angles between H-9 and the two H-8 protons are about 30 and 90°. The 7-*epi* form cannot maintain structure **6** and must invert to the opposite envelope structure or, more likely, the energetically similar half-chair form **7** since the observed couplings for H-9 are 3.6, 3.6 and 2 0 Hz Thus $^3J$(H-8, H-9) is 3.6 Hz for both H-8 protons indicative of equal torsion angles of about 60°. The third coupling is $^4J$(H-1a, H-9), with a similar value in both epimers since the conformation at C-1 does not change. Proton H-1e will not couple to H-9 since it is nearly co-planar with the double bond. These observations on the couplings at H-9 and the lack of substantial changes elsewhere in the spectrum support our assignment of a 7-*epi*-debneyol (**2b**) structure to this species. Furthermore they establish a 7$\beta$ configuration for debneyol (previously assigned on the basis of NOE measurements [7]) and confirm that the double bond is C-9 (10) as suggested from mass spectral evidence [6] and decoupling experiments on related compounds [7].

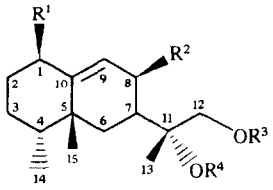
The 12-*O*-acetyl derivatives of **2a** and **2b** were also obtained as a 7$\alpha$,7$\beta$ mixture (about 1:10) the minor isomer (**8b**) giving the characteristic olefinic multiplet (*dt*) at $\delta$5.34 Methyl group chemical shifts are given in Table 1 but the H-12, H-12′ protons could not be easily distinguished in the presence of major 7$\beta$ epimer (**8a**).

Treatment of a portion of the **2a**/**2b** (10:1) sample with acetone in the presence of acid gave the corresponding 11,12-*O*-isopropylidene derivatives **9a** and **9b**. Chemical shifts for the 7$\alpha$ (**9b**) and 7$\beta$ (**9a**) isomers are similar to

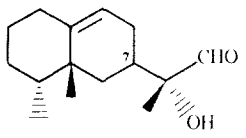
Table 1 Selected $^1$H NMR data for

| H | 2a | 2b | 8a | 8b | 9a | 9b | 11a |
|---|---|---|---|---|---|---|---|
| 1 | | | | | | | |
| 3 | | | | | | | |
| 9 | 5 49 *dt* (6 4, 1 8) | 5 35 *dt* (3 6, 2.0) | 5 47 *dt* | 5 34 *dt* | 5 49 *dt* | 5 35 *dt* | 5 35 *dt* |
| 12 | 3 40 *d* (11 0) | 3 35 *d* (11 0) | 3 97 *d* | † | 3 69 *d* | 3 64 *d* | 9 56 |
| 12′ | 3 56 *d* (11 0) | 3 51 *d* (11 0) | 4 05 | † | 3 84 *d* | 3 81 | |
| 13 | 1 09 *s* | 1 06 *s* | 1 13 *s* | † | 1 21 *d* (0 4) | 1 18 *s* | 1 14 *s* |
| 14 | 0 97 *d* (7 0) | 0 96 *d* (7 0) | 0 96 *d* | 0 94 *d* | 0 98 *d* | 0 96 *d* | 0 93 *d* |
| 15 | 1 17 | 1 14 | 1 16 | 1 10 | 1 17 | 1 14 | 1 29 |
| 17 | | | | | 1 36 | 1 34 | |
| 17′ | | | | | 1 43 | 1 41 | |

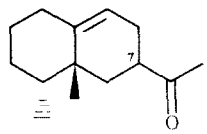
*Chemical shifts ($CDCl_3$, relative to TMS) are given for all assignable protons Useful
†Obscured by major isomer
‡Acetyl groups




| | R¹ | R² | R³ | R⁴ | |
|---|---|---|---|---|---|
| **2a** | H | H | H | H | 7β |
| **2b** | H | H | H | H | 7α |
| **4** | OH | H | H | H | 7β |
| **5** | H | OH | H | H | 7β |
| **8a** | H | H | H | Ac | 7β |
| **8b** | H | H | H | Ac | 7α |
| **9a** | H | H | $CMe_2$ | | 7β |
| **9b** | H | H | $CMe_2$ | | 7α |
| **13** | OAc | H | Ac | H | 7β |
| **14** | H | OAc | Ac | H | 7β |
| **15** | OAc | H | H | H | 7β |




**11a** 7β
**11b** 7α




**12a** 7β
**12b** 7α

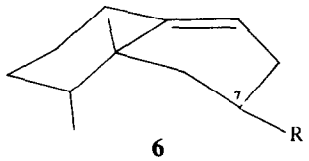
those of the parent diols (Table 1) except that one of the protons on C-12 has a splitting corresponding to $^4J$(H-12′, H-13)=0.4 Hz. This coupling is also evident in the methyl group and corresponds to an interaction with the synclinal proton as shown in **10**. In fact H-12′ has a significantly greater line width in **2a/2b** and the monoacetates **8a/8b** indicating a similar but smaller stereospecific coupling in the diol. Evidently debneyol also has a side chain conformation similar to **10** It is notable that the methyl groups at C-2 of the dioxolan ring are mutually coupled, $^4J$(H-17, H-17′)=0 6 Hz, in both the 7α and 7β forms and that in the 7α(*epi*) form these groups show similar upfield shifts to the other methyl groups (C-13, C-14, C-15) supporting the contention that we are dealing with stereoisomerism at C-7, rather than a site more remote from the side chain The $^{13}$C NMR spectrum of **9a/9b** was unexceptional showing significant downfield shifts for C-7, C-11, C-12, and C-13 as expected (see Experimental).

A portion of the **2a/2b** (10 1) sample was oxidized with ruthenium-(II)-tris-(triphenylphosphine)-dichloride [$Ru(PPh_3)_3Cl_2$] to afford a mixture of two epimeric aldehydes, debneyal (**11a**) and 7-*epi*-debneyal (**11b**) (10 1) and two epimeric ketones, 12-nordebneyone (**12a**) and 7-*epi*-12-nordebneyone (**12b**) (10 1) This is in contrast to the action of $CrO_3$–pyridine or activated $MnO_2$ as oxi-

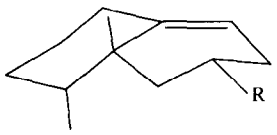
debneyol and related compounds*

| 11b | 12a | 12b | 4 | 15 | 13 | 5 | 14 |
|---|---|---|---|---|---|---|---|
| | | | 4 25 *t* (2.9) | 5.26 *dd* | 5 26 *dd* | | |
| | | | | | | 4 45 *dt* (6 6, 1 8, 1 6) | 5.31 |
| 5 37 *dt* | 5 60 *dt* | 5 41 *dt* | 5 83 *dd* (7 1, 1 3) | 5.96 *dd* | 5.98 *dd* | 5 80 *dd* (6 6, 1 8) | 5 83 |
| 9.52 | | | 3 41 *d* | 3 42 *d* | 3 99 *d* | 3 70 *d* | 4 06 *d* |
| | | | 3.56 *d* | 3 57 *d* | 4 04 *d* | 3.30 *d* | 4 03 *d* |
| 1.12 *s* | 2.17 *s* | 2 21 *s* | 1 11 | 1 10 | 1 15 | 1 25 *s* | 1 25 *s* |
| 0.89 *d* | 1 00 *d* | 0.89 *d* | 0 95 *d* | 0 97 *d* | 0 96 *d* | 0 95 *d* | 0 94 *d* |
| 1.18 | 1.19 | 1 26 | 1 37 | 1 26<br>2 02 *s*‡ | 1 27<br>2.02 *s*‡<br>2 11 *s*‡ | 1 31 *s* | 1 27 *s*<br>2 04 *s*‡<br>2 08 *s*‡ |

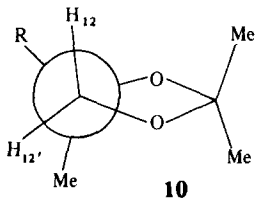
couplings are given in parenthesis for representative samples ($J$ in Hz)




**6**




**7**




**10**

dants on **2a** which only give **12a** [6, 7]. $Ru(PPh_3)_3Cl_2$ has previously been employed to selectively oxidise primary alcohols in the presence of secondary alcohols [10] and we have used it successfully to synthesize both [15-$^3$H]lubimin and 10-*epi*-[15-$^3$H]lubimin from 15-[15-$^3$H]dihydrolubimin [11]

The chemical shifts in both **11a/11b** and **12a/12b** follow the established pattern (Table 1). Exceptionally the bridgehead methyl singlet (H-15) is downfield in the 7α ketone relative to the 7β form. The olefinic proton in **11b** and **12b** shows the same coupling pattern ($dt$, $J = 3\,6$, 3.6, 2 0 Hz) as observed for **2b** indicating that these *epi* isomers maintain the half-chair conformation **7**. Treatment of the ketones **12a/12b** with sodium hydroxide in aqueous ethanol for 30 min resulted in partial epimerisation at C-7. From the $^1$H spectrum the 7α epimer (**12b**) was about 21% of the mixture (compared to 9% initially) This further confirms our assignment of the minor isomer to a 7α form since no other chiral centre can epimerise under these conditions After one week in the presence of base, the 7α epimer was 85% of the mixture, indicating that the natural 7β form of debneyol (**2a**) is probably *ca* 4 kJ/mol less stable than the other epimer. It should be noted that in addition to the identified 7-*epi*-debneyol (**2b**) several other minor species were present in the original debneyol fraction

The $^1$H NMR spectrum of the capsidiol TLC fraction revealed the presence of two minor species (in the ratio 1 1) each with absorptions characteristic of an endocyclic double bond (H-9), a dihydroxyisopropyl group (H-12, H-12', H-13) and a secondary alcohol These trihydroxy compounds were identified as 1-hydroxydebneyol (**4**) and 8-hydroxydebneyol (**5**) on the basis of the following evidence Acetylation of the capsidiol fraction and subsequent TLC gave capsidiol diacetate ($^1$H NMR in agreement with published data [12]) and a slower running component which was a mixture of the two diacetates **13** and **14** in the ratio 3 : 2 The $^1$H NMR data for this mixture (Table 1) were in agreement with acetylation at *O*-12 in both species since H-12 and H-12' are shifted downfield, similar to debneyol acetate (**8a**). Acetylation of the secondary alcohol in both species is evident in the downfield shifts for the CHOAc group in each case Deacetylation of the mixture of diacetates followed by TLC gave a mixture of the two triols (**4/5**, 3 : 7) and a pure sample of 1-acetoxydebneyol (**15**) These changing proportions are in keeping with substantial differences in the rates of acetylation/deacetylation of the secondary hydroxyl groups, one being more hindered than the other. The position of the endocyclic hydroxyl group in 1-hydroxydebneyol (**4**) is established by H-1 at δ4 25 (cf H-1 in **2a** at 4 36) and H-9 at δ5.83 (cf H-9 in **2a** at 5.93).

Furthermore, the absence of allylic coupling [$^4J$(H-1a, H-9) = 1.8 Hz in debneyol (**2a**)] establishes the configuration at C-1 as $\beta$-hydroxy. Similarly the absence of a small vicinal coupling at H-9 in 8-hydroxydebneyol (**5**) indicates a $\beta$-hydroxyl in this case also The position of the endocyclic hydroxy group in **5** accounts for the greater separation of H-12 and H-12', the loss of a coupling to H-7 (*dt*, 2 0), and a lower reactivity under acetylation

## DISCUSSION

The presence of 7-*epi*-debneyol (**2b**) in the debneyol (**2a**) sample isolated from cell suspension cultures of *N tabacum* var White Burley is of interest because this compound was not detected in samples of debneyol obtained from virus inoculated leaves of *N debneyi* [6] although a minor isomer of debneyol was reported to be present in extracts of suspended callus cultures of *N tabacum* (unknown var) elicited with cellulase [7]. A somewhat similar situation occurs with the vetispiranes lubimin and 3-hydroxylubimin Both compounds have been isolated along with their C-10 epimers from *Solanum tuberosum* [13–15] whereas only lubimin and 3-hydroxylubimin have been obtained from *D stramonium* [2 and personal observations]. In addition, 2-*epi*-lubimin has been obtained from *S. tuberosum* [16] but not from any other source This would seem to imply that several of the enzymes responsible for the formation of these compounds show less stereospecificity in some plants than in others.

The currently postulated biosynthetic pathway leading to capsidiol formation envisages the direct precursor of capsidiol to be a *cis*-eudesmanoid intermediate which is converted to capsidiol by a concerted mechanism known to involve both a methyl migration from C-10 to C-5 [18] and a hydride shift from C-5 to C-4 [19] Recent work has confirmed that the eremophilene structure of debneyol arises through methyl migration from C-10 to C-5 in an identical manner to that observed in the biosynthesis of capsidiol and that the primary hydroxyl group of debneyol is on the C atom equivalent to C-12 in capsidiol [20]

Based on the above evidence, the observation that both compounds accumulate at the same time in cellulase-elicited cell suspension cultures of *N. tabacum* [9] and the fact that both compounds have identical carbon skeletons with the same stereochemical features, we would like to propose that the hydrocarbon 4-*epi*-eremophila-9,11-diene (**16**) is the common precursor of both capsidiol (**1**) and debneyol (**2a**) (Fig 1) Compound **16** is the C-4 epimer of aristolochene previously obtained from the roots of *Aristolochia indica* [4, 21]

In support of this proposal we have shown that a hydrocarbon with the TLC properties expected of **16** is the major radioactive compound synthesized from either [1-$^{14}$C]isopentenyl pyrophosphate or [1-$^3$H]farnesyl pyrophosphate by a crude, cell-free system prepared from cells of cellulase-elicited tobacco cultures Furthermore, when NADPH is co-administered with [1-$^{14}$C]isopentenyl pyrophosphate to the cell-free system there is incorporation of radioactivity into capsidiol (**1**), and debneyol (**2a**) and another unknown (with the TLC properties expected for a monohydroxy-**16**) the magnitude of which is matched by a decrease (70%) in the amount of radioactivity incorporated into the hydrocarbon [9] The most obvious route for the formation of **16** from farnesyl pyrophosphate is via germacrene A and a eudesmane carbocation (as its enzyme-bound equivalent). The experimental evidence obtained from studies with cell-free systems (see above) from $^{18}O_2$ studies on the biosynthesis of rishitin [22] indicate that the conversion of **16** into capsidiol (**1**) and the introduction of at least one of the two hydroxyl groups in debneyol (**2a**) will be by way of NADPH- and $O_2$-dependent hydroxylation reactions The vicinal hydroxyl groups in the side chain of debneyol could be introduced at C-11 and C-12 of the isopropenyl side chain of **16** by epoxidation (NADPH- and $O_2$-dependent) followed by hydration, by hydration followed by monohydroxylation (unlikely since no hydrated **16** is formed in the NADPH-deficient incubations, see above) or by some more complicated route

It seems likely that both 1-hydroxydebneyol (**4**) and 8-hydroxydebneyol (**5**) belong to a family of hydroxylated compounds which can arise from **16** (Fig 1) However, on the basis of its structure and stereochemistry, 8$\alpha$-hydroxydebneyol (**5**) would appear to be formed from debneyol (**2a**) and is probably the direct precursor of cyclodebneyol (**3**). However, it is not clear at what level of the proposed pathway leading from farnesyl pyrophosphate to debneyol that the $\alpha$-configuration at C-7 needed to account for 7-*epi*-debneyol (**2b**) is introduced

Work is in progress to identify the two unknowns formed in the cell-free systems and also to establish the origin of the oxygen atoms of both capsidiol (**1**) and debneyol (**2a**).

## EXPERIMENTAL

$^1$H and $^{13}$C NMR spectra were recorded at 270 05 and 67 9 MHz respectively All TLC was performed on 0 5 mm rhodamine 6G-impregnated silica gel G with the following solvents A = EtOAc–cyclohexane (1 1), B = EtOAc–*i*PrOH (9 1), C = petrol (40–60°)–$Et_2O$ (20 1) All reagents were Analar Solvents were redist before use

*Plant tissue cultures* Cell suspension cultures of *Nicotiana tabacum* L var White Burley were grown at 25° with constant illumination and agitation (110 rpm) in 250 ml conical flasks (100 ml media) on Gamborg's B5 media [23] containing 2,4-D (1 mg/l), kinetic (0 1 mg/l) and sucrose (20 g/l) They were maintained by sub-culture every 7 days Large scale cultures (800 ml media in 2 5 l conical flasks) were set up by inoculating the flasks with the whole of a small culture (*ca* 40 g fr wt of cells) and were grown under the same regime The large cultures achieved a biomass of *ca* 320 g fr wt in 7 days

*Elicitation and isolation of phytoalexins* Four large cultures (6 days old) were each treated with 2 4 mg cellulase (Ex *Trichoderma viride*, Sigma) for 16 hr The cultures (*ca* 1 9 kg fr wt) were harvested by filtration through Miracloth and the media was extracted with $Et_2O$ (3 × equal vol) The pooled $Et_2O$ extracts were reduced to dryness *in vacuo* TLC (solvent A) gave 40 mg of a fraction containing debneyol (**2a**)/7-*epi*-debneyol (**2b**) and acetosyringone [9] at $R_f$ 0 30 and 57 mg of a fraction containing capsidiol (**1**), 1-hydroxydebneyol (**4**) and 8-hydroxydebneyol (**5**) in a ratio of approximately 8 1 1 at $R_f$ 0 12

*Purification and characterisation of* **2a**/**2b** The crude fraction obtained above was dissolved in a few ml of EtOH and treated with a small amount of $NaBH_4$ to reduce the acetosyringone After 15 min the mixture was diluted with $H_2O$ and extracted with $Et_2O$ (3 × vol) After reduction of the solvent *in vacuo*, the residue was purified by TLC (solvent A) to give 19 mg **2a**/**2b** (10 1) at $R_f$ 0 30 The EIMS spectra of the mixture of **2a** and **2b**

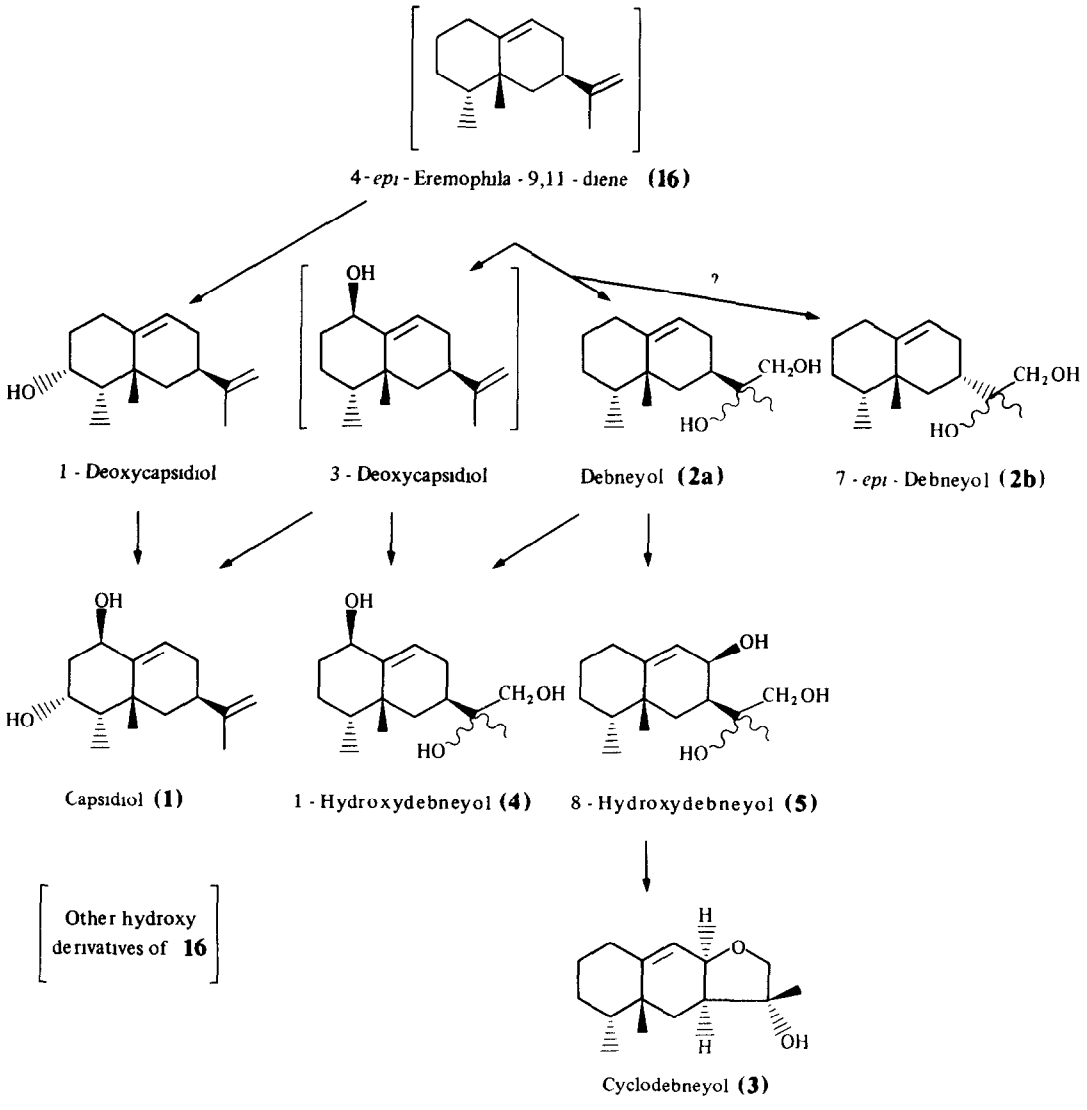



Fig 1 Suggested biosynthetic relationships between eremophilenes isolated from *N tabacum*

was identical to that reported for **2a** [6] with the exception of being able to observe the $[M]^+$ ion at *m/z* 238 (0 6%). $^1H$ NMR of the mixture (see Table 1) indicated that the C-7 epimer accounted for *ca* 9% of the material. Repeated TLC (solvent A) failed to resolve the two epimers but did enable an enriched fraction (1 mg, **2a/2b**, 1 · 1) to be obtained from the leading edge of the sample Since the amount of this material was so small the chemical derivatisations were performed on the original unenriched material

12-O-*Acetyldebneyol* (**8a**) *and* 7-epi-12-O-*acetyldebneyol* (**8b**) 5 mg of **2a/2b** (10 · 1) was acetylated overnight with $Ac_2O$ (200 μl) and pyridine (100 μl) [6, 7]. After the addition of EtOH (200 μl), TLC (solvent A) afforded **8a** and **8b** (5.7 mg, 10 · 1) at $R_f$ 0 54 The EIMS spectra was very similar to that reported for **8a** [6] with the exception of being able to observe the $[M]^+$ ion at *m/z* 280 (0 3%), the $[M-H_2O]^+$ ion at *m/z* 262 (1.3%) and the $[M-C_2H_4O_2]^+$ ion at *m/z* 220 (0.3%)

11,12-O-*Isopropylidenedebneyol* (**9a**) *and* 7-epi-11,12,-O-*isopropylidenedebneyol* (**9b**) 5 mg of **2a/2b** (10 . 1) was treated with 500 μl of dry acidified $Me_2CO$ (prepared from 5 ml $Me_2CO$ and 100 μl conc HCl) for 1 hr at room temp. TLC (solvent A) afforded **9a** and **9b** (3 8 mg, 10 · 1) at $R_f$ 0.71 and unreacted starting material (0 8 mg) at $R_f$ 0 30 [25]. EIMS *m/z* (rel int ) 278 $[M]^+$ (0 6), 263 (7), 260 (0 6), 220 $[M-C_3H_6O]^+$ (25), 205 (19), 189 (15), 162 (29), 147 (25), 133 (17), 115 (100), 105 (60), 91 (55), 79 (31) and 55 (58) $^{13}C$ NMR ($CDCl_3$): 17 54 (C-14), 22.05 (C-13), 22 52 (C-2), 22 63 (C-8), 27 03 (C-17), 27 64 (C-17'), 30 32 (C-3, C-15), 32 11 (C-1), 38 63 (C-5), 39 57 (C-6), 41 54 (C-7), 41.63 (C-4), 73 31 (C-12), 83.48 (C-11), 108.99 (C-16), 120 04 (C-9) and 141.73 (C-10)

*Debneyal* (**11a**)/7-epi-*debneyal* (**11b**) and 12-nor-*debneyone* (**12a**)/7-epi-12-nor-*debneyone* (**12b**) 5 mg of **2a/2b** (10 · 1) was stirred with 24.4 mg (1 2 mol excess) of ruthenium(II)-tris-(triphenylphosphine)-dichloride (Thiokol Chemicals Ltd Coventry [9]) in $C_6H_6$ (650 μl) at room temp for 3 hr The reaction mixture was spotted directly onto a TLC plate which after development with solvent A gave unreacted starting material (2 4 mg) at $R_f$ 0 30 and a single higher pink band (visualized

under $UV_{254}$ light) at $R_f$ 0·67. Re-TLC of the higher band (solvent C) afforded **11a/11b** (*ca* 0·5 mg, 10:1) at $R_f$ 0·06 and **12a/12b** (*ca* 0·5 mg) at $R_f$ 0·14. **11a/11b**: EIMS *m/z* (rel. int.): 236 $[M]^+$ (0·6), 218 $[M-H_2O]^+$ (1), 207 $[M-CHO]^+$ (6), 206 (8), 189 $[M-H_2O-CHO]^+$ (11), 175 (3), 162 (96), 147 (29), 133 (21), 119 (29), 105 (91), 91 (100), 81 (86), 71 (41), 67 (43), 58 (54), and 55 (83); IR $\nu_{max}^{film}$ $cm^{-1}$: 3480 (–OH), 2790 (–CHO), 1728 (–CHO), 858 (C=C); $^1H$ NMR: see Table 1.

**12a/12b**: EIMS data were identical to reported values [6]; $^1H$ NMR: see Table 1. Due to the volatile nature of both the aldehydes and the ketones it was necessary to adopt appropriate precautions when handling samples of these materials.

*Epimerisation of* **12a/12b**. 4 mg of **2a/2b** (10:1) in $Et_2O$ (500 μl) and $H_2O$ (1 ml) was treated with Jones reagent (100 μl) for 3 hr. The reaction mixture was diluted with $H_2O$ (3 ml) and extracted with $Et_2O$ (3 × vol.). The $Et_2O$ extracts were combined, washed with 5% $Na_2S_2O_3$ (1 × vol.), 5% $NaHCO_3$ (1 × vol.) and $H_2O$ (1 × vol.) (GC analysis showed that the reaction had gone to completion). The solvent was removed under $N_2$ and the residue dissolved in EtOH (200 μl). 2 M NaOH (100 μl) was then added. After 30 min the mixture was diluted with $H_2O$ and extracted with $Et_2O$. TLC (solvent A) gave **12a/12b** as a 4:1 mixture. Further treatment of the 4:1 mixture with base for 7 days at 4° gave **12a/12b** as a 3:17 mixture.

*Separation and characterization of capsidiol* (**1**), *1-hydroxydebneyol* (**4**) *and 8-hydroxydebneyol* (**5**). Direct TLC of the sample containing the three compounds in either solvent A or B did not resolve any of the components. However, after acetylation of 8 mg of the mixture overnight of room temp. with $Ac_2O$ (200 μl) and pyridine (100 μl) followed by the addition of EtOH (200 μl), TLC (solvent A) gave pure capsidiol diacetate ($R_f$ 0·68) which was identical in all respects (TLC, GC, EIMS, IR, $^1H$ NMR) [12] to a sample prepared from pure capsidiol (**1**) obtained from *Capsicum annuum* [24] and a mixture of the diacetates **13** and **14** (6·4:1) at $R_f$ 0·55. Treatment of the mixture of **13** and **14** with 2 M NaOH (100 μl) in EtOH (200 μl) and pyridine (50 μl) for 30 min followed by extraction with $Et_2O$ and TLC (solvent A) gave pure 1-acetoxydebneyol (**15**) (*ca* 0·5 mg, $R_f$ 0·24) and a mixture of 1-hydroxydebneyol (**4**) and 8-hydroxydebneyol (**5**) (*ca* 0·5 mg, 3·7:1, $R_f$ 0·16). **15**: EIMS *m/z* (rel. int.): 296 $[M]^+$ absent, 278 $[M-H_2O]^+$ (0·3), 236 $[M-C_2H_4O_2]^+$ (3), 218 $[M-H_2O-C_2H_4O_2]^+$ (5), 205 (11), 200 $[M-2H_2O-C_2H_4O_2]^+$ (2), 187 (13), 162 (15), 159 (17), 145 (12), 133 (7), 119 (17), 105 (29), 91 (19), 83 (28), 75 (25), 67 (8), 57 (17) and 43 (100). $^1H$ NMR: see Table 1. **4** and **5**: EIMS *m/z* (rel. int.): 254 $[M]^+$ (0·1), 236 $[M-H_2O]^+$ (2), 223 (7), 218 $[M-2H_2O]^+$ (3), 205 (10), 200 $[M-3H_2O]^+$ (0·6), 187 (4), 180 (6), 161 (14), 147 (11), 135 (8), 123 (21), 105 (42), 91 (28), 83 (60), 75 (16), 67 (13), 57 (21), and 43 (100). $^1H$ NMR: see Table 1.

*Analytical GC*. The conditions used for GC analysis have been described previously [25] except that the column temp. used was 175°. The $RR_t$ values [Me stearate ($R_t$ 5·2 min) = 1] were as follows: 12-*nor*-debneyone 0·35, 11,12-*O*-isopropylidenedebneyol 0·46, debneyal 0·70, 12-*O*-acetyldebneyol 1·08, debneyol 1·70, capsidiol 2·07, 8-hydroxydebneyol 4·71, 1-hydroxydebneyol 7·35.

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