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CEMBRENEDIOLS IN THE CURING OF TOBACCO. X-RAY CRYSTAL STRUCTURES OF β-CEMBRENEDIOL AND α-CEMBRENEKETOL

MICHAEL J. BEGLEY, LESLIE CROMBIE, DESMOND MCNAMARA, DAVID F. FIRTH, SUE SMITH and PETER C. BEVAN*

Department of Chemistry, University of Nottingham, Nottingham, NG7 2RD, U.K.; *Research and Development Centre, B.A.T. (U.K. and Export) Ltd., Regents Park Road, Southampton, SO9 1PE, U.K.

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; α- and β-cembrenediols; isotopic labelling; curing; X-ray crystal structures; α-cembreneketol.

Abstract—[U-^{14}C]-α-and β-Cembrenediols (CBDs) have been prepared photosynthetically using $^{14}CO_2$ assimilated by the tobacco plant. Isotopic hydrogen can be introduced into α-CBD by oxidation to the 6-ketone followed by reduction by borohydride (deuteride or tritide) modified by cerium trichloride to suppress 1,4-reduction. [6β-^{3}H]-4α,6α-CBD having natural stereochemistry and its [6α-^{3}H]-epimer thus formed (ratio ~ 3:1) are separable by HPLC. Used for the 4β,6α-CBD series however, the method gives mainly unnatural [6α-^{3}H]-4β-,6β-CBD. [U-^{14}C]-Labelled α- and β-CBDs were applied to *Nicotiana tabacum* leaf and subjected to a laboratory simulation of the flue-curing process. Most of the radioactivity remained in surface products (~ 80%) with 2.5–7.5% migrating into the leaf but remaining extractable. Losses as volatiles amount to 10–17% but the amount of ^{14}C fixed by leaf tissue remains very small. Only about 10% of the original α-CBD survives intact during curing, though with the more stable β-CBD this rises to 40%. A substantial amount of products more polar than the CBDs is formed (probably epoxides, triols etc.) along with less polar materials probably arising from dehydration etc. A single crystal X-ray structure for β-CBD is reported, but the α-isomer was not amenable to direct methods treatment. However, an X-ray structure for the α-ketol was achieved and it is shown by F.T. IR spectroscopy of α-CBD in dilute carbon tetrachloride solution that it possesses an intramolecular hydrogen bond which is not present in β-CBD epimer: this has significant conformational consequences. 2-D-^{13}C/^{1}H Correlation NMR spectra for α- and β-CBDs are recorded.

INTRODUCTION

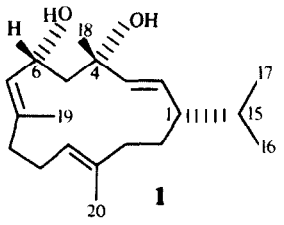
It is generally recognised that the characteristic flavour and aroma [1,2] of Virginia and Burley tobaccos depend to a significant extent on degradation products from two important tobacco-leaf cembrenoids, (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-4,6-dihydroxycembra-2,7,11-triene (α-cembrenediol or α-CBD) (**1**) and its (4-*R*)-epimer (β-cembrenediol or β-CBD) (**2**) [3,4]. Oriental, Greek and Turkish tobaccos on the other hand contain labdanoids such as (12*Z*)-abienol (**3**) and (13E)-labden-8,15-diol (**4**) (and many relatives) which contribute to the flavour and aroma [5], although cembrenoids are also often present [2]. Cured tobacco contains a very large range of compounds [5] including a host of smaller odorous terpenic fragments derived from (**1**) and (**2**), and (**5**) shows one of the major seco-cleavages giving (**6**), from which originates a cascade of smaller fragments [7]. Cleavage (**7**) is represented by (**8**), found in tobacco smoke [8]. Virginia tobacco is normally flue cured and the aim of the present investigation, which is preliminary in character, has been to devise a methodology for studying the fate of tobacco cembrenoids during the curing process. X-ray, F.T. IR and 2D-NMR investigation of the CBDs is also reported.

RESULTS AND DISCUSSION

Isotopic labelling of cembrenediols

For following the fate of the α- and β-CBDs during curing [U-^{14}C]-labelling is the most useful, though for more detailed study of the mechanism of formation of fragments, specifically positioned ^{14}C- and ^{3}H- along with ^{18}O- labels will be required. For [U-^{14}C]-(**1**) and (**2**) a photosynthetic method using $^{14}CO_2$ generated from Ba $^{14}CO_3$ was used. A young plant *ca* 18″ high was employed and photosynthesis was allowed to proceed for 6 hr before isolation of the leaf surface extractable material by dipping in dichloromethane. The leaf surface extract was partitioned between methanol–water (9:1) and hexane (cf. Table 1). The polar fraction (~ 35% of the initial extract) was then purified by C_{18}-reversed phase HPLC, eluting with methanol–water (3:1). [U-^{14}C]-α- and β-CBDs were successfully separated and isolated by these means, together with a fraction containing materials more polar than α-CBD which was not examined further. Both CBDs were obtained crystalline from hexane or pentane and this [U-^{14}C]-material, crystallized to constant count, was used in the curing experiments below. The majority (72.4%) of the ^{14}C found in the leaf-surface extract is contained in the polar material (Table 1). Of this polar material, α-CBD carries 38.5% and β-14.7% of the radioactivity. The incorporation of ^{14}C into the CBDs from the $^{14}CO_2$ administered was 0.19% for the α- and 0.07% for the β-, i.e. a ratio of 2.7:1. Since the usual proportion of α-to β-CBD is 2.6:1 in tobacco leaf extract obtained by dipping, this suggests that the ratio of formation of two compounds is rather similar to the ratio of final concentrations found.

For specific labelling of α- and β-CBDs with isotopic hydrogen at C-6, pure crystalline 'cold' CBDs were re-



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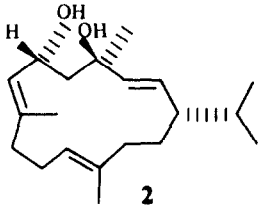

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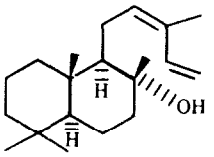

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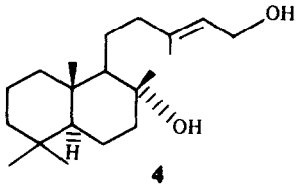

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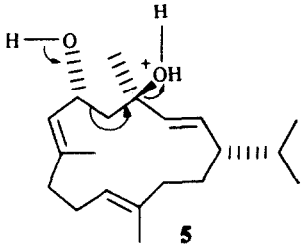

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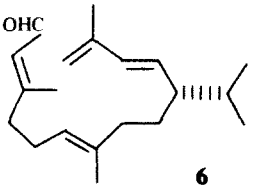

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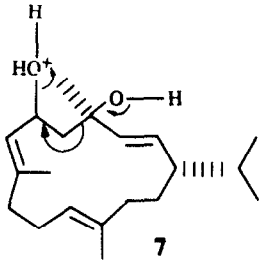

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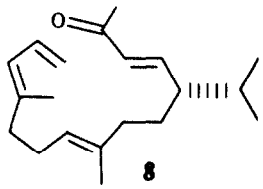

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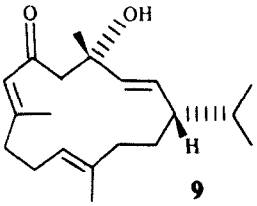

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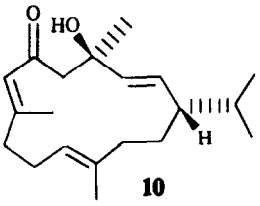

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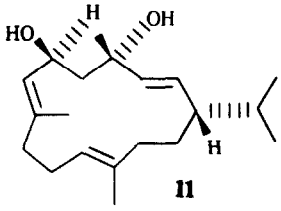

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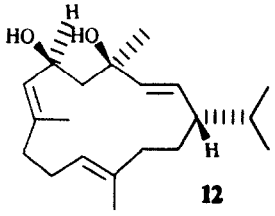

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Table 1. Isolation of [U-^{14}C]-α- and β-cembrenediols from tobacco leaf after photosynthesis in $^{14}CO_2$

	Total activity (μCi)	Specific activity (μCi/g)	% Activity*
Leaf surface extract	34.8	173.1	
After solvent distribution:			
Non-polar part	8.5	65.5	
Polar part	25.2	354.3	
Aqueous washings	0.9		
After HPLC:			
α-CBD (1)	9.7		27.9
β-CBD (2)	3.7		10.6
More polar than CBDs.	12.8	325.2	36.8
Residue	0.3		0.7

* Relative to leaf surface extract activity.

quired and these were isolated from tobacco leaf-surface extract by partition and HPLC [9]. Labelling was introduced by an oxidation–reduction cycle. Using chromium trioxide–pyridine, α-CBD was oxidized to the 6-ketone (**9**) in 80% yield though yields were poorer (49%) in the β-CBD case, leading to **10**. Manganese dioxide as oxidant however gave improved yields of 95 and 91% respectively. Both ketones have been isolated from cured tobacco [10, 11]. Reduction of enone (**9**) with sodium borodeuteride did not proceed satisfactorily as the expected 1,2-reduction products 6-deuterio- (**1**) and 6-deuterio-(**11**) were formed in only 30% yield the remainder being products of 1,4-reduction. Further experimentation using sodium borohydride showed that addition of a lanthanide salt, e.g. cerium trichloride [12], greatly improved the 1,2-specificity of the reduction and ketone **9** was reduced in 10 min to a mixture of (**1**) and (**11**) in 62% yield. Using cerium trichloride modified sodium borotritide a similar reduction of (**9**) gave a mixture of the expected labelled diols (70%) which were separated by HPLC to give the labelled natural [6β-^{3}H]-4α,6α-CBD (cf. **1**) and the unnatural [6α-^{3}H]-4α,6β-CBD (cf. **11**). A similar procedure was attempted for the radiolabelling of (**2**). Cerium trichloride modified reduction of ketone (**10**) with sodium borohydride proceeded in 88% yield to give the mixture of required diols but in this case the reduction was much less favourable to production of the diol of the natural series. The 4β-,6α-(**2**) and 4β-,6β-(**12**) isomers were produced in a ratio of less than 1:9.

The effects of flue-curing on cembrenediols

Our experiments on the fate of α- and β-CBDs during the curing of tobacco leaf were carried out using [U-^{14}C]-specimens. Each radiolabelled specimen was dissolved in acetone and applied by spotting over the surface of a large freshly harvested tobacco leaf (*Nicotiana tabacum* var. McNair). The leaves were suspended in a laboratory-scale curing oven and flue-cured for six days. Conditions used are summarized in graphical form in Fig. 1. Initially during curing the leaves are subjected to humidity but at fairly low temperatures. In this leaf-yellowing phase starch is converted into sugars, but without causing oxidation of leaf components (e.g. polyphenols which cause browning of the leaf). During colour fixation the temperature is gradually raised and the humidity lowered. Air is circulated through the curing oven and the temperature increased, resulting in leaf drying but avoiding excessive oxidation (and hence unwanted colour changes). The temperature is raised again to complete lamina, and then stem, drying, humidity being increased to avoid a brittle leaf.

The radiochemical situation after curing is shown in Table 2. Most of the radioactivity (~80%) remains on the leaf surface with 2.5–7.5% having migrated into the leaf and remaining in extractable form. The actual amount of [^{14}C] fixed in the leaf tissue (determined by combustion to $^{14}CO_2$) is very small but 10–17% has been lost as volatile material.

Further investigation (Table 3) of the leaf-surface extract of the cured leaves shows that it contains less than 2% of non polar material. Only about 9.6% of the originally applied α-CBD remain on the leaf at the end of the curing process, but some 38.5% β-CBD survives. This is explained by the greater stability of β-CBD towards exposure to air and heat: α-CBD is known to be far less stable in these respects. A large percentage of both isomers is converted into compounds more polar than CBDs and this fraction probably contains epoxides, triols and similar chemical species. A lesser amount is converted into products less polar than the CBDs, more being formed by the α-CBD than the β-. This material probably contains compounds of lower oxygenation than the CBDs formed by dehydration etc. during curing, and these fractions now await more detailed chemical investigation. Whilst our work is of a preliminary character, the results reported open the way to a more detailed understanding of the part played by the chemistry of the α- and β-CBDs in the production of Virginia tobacco by the flue-curing process.

During the course of the studies reported in this and the preceding paper [9], we have carried out X-ray crys-

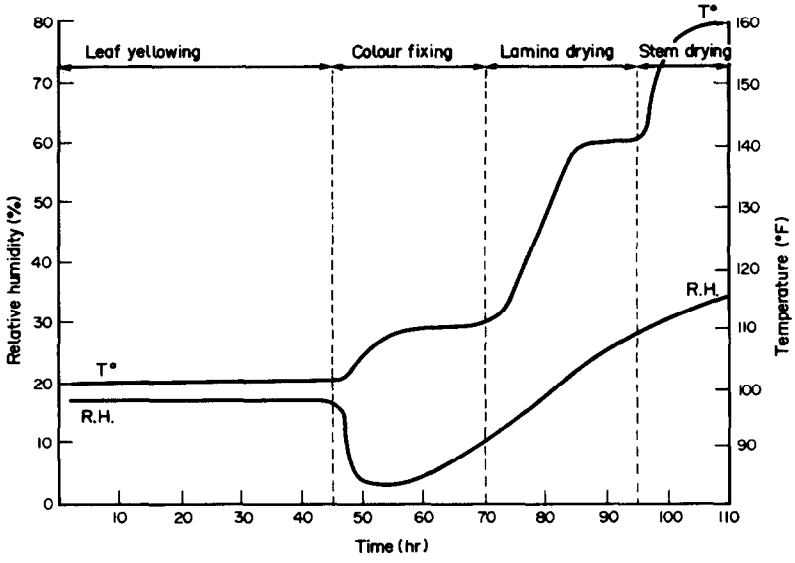

Fig. 1. Conditions used for tobacco leaf curing.

Table 2. Distribution of [^{14}C]-labelled products formed during curing of tobacco leaf surface treated with [U-^{14}C]-α- and -β-cembrene-4,6-diols

	Leaf 1 α-CBD	Leaf 2 β-CBD	Leaf 3 β-CBD
[^{14}C]-Activity applied %	100*	100†	100†
After curing:			
Leaf surface extract [^{14}C] %	81.4	79.0	81.3
Leaf interior extract [^{14}C] %	7.7	3.5	2.5
Remaining leaf tissue [^{14}C] %‡	0.5	0.6	16.2
Activity lost as volatiles %§	10.4	16.9	

*4.084 μCi.
†1.427 μCi.
‡By combustion.
§By difference.

Table 3. Polarity distribution of [^{14}C]-labelled products in tobacco leaf surface extract

		Leaf 1 α-CBD	Leaf 2 β-CBD
Original leaf surface extract [U-^{14}C]-	%	100	100
Non-polar fraction	%	1.6	1.7
Polar fraction	%	98.4	98.3
Material less polar than CBDs	%	18.2	11.4
Recovered CBD	%	11.7	48.7
Material more polar than CBDs	%	68.5	38.2

tallographic and spectroscopic studies on the cembrenediols. β-Cembrenediol crystallized from hexane in the trigonal space group $P3_121$ with six molecules per unit cell. The X-ray structure was solved by direct methods using diffractometer data (see Experimental) to $R = 9.66\%$ over 949 observed reflections. The rather high final R value is partly due to comparatively weak data from the small-sized crystal, and partly due to disorder, or high thermal motion, in the non-α-hydroxy-alkene (C-9) and isopropyl (C-18) regions of the molecules where packing forces are presumably weak. [The X-ray crystallographic numbering employed is shown in (**13**) (Fig. 2) and is not the same as the chemical numbering (cf. **2**)]. The resulting structure for β-CBD is shown in Fig. 3. There is an earlier report of an incomplete X-ray study of β-CBD in the literature (described as β-4,8,13-duvatriene-1,3-diol, coded C-2) though little detail and no picture is given [13].

α-Cembrenediol crystallized from hexane as small orthorhombic crystals in space group $P2_12_12_1$. However, the large volume of the unit cell contained 36 molecules, which requires nine crystallographically independent molecules in the asymmetric unit, and a direct methods solution was not attempted. Instead, the corresponding α-ketol [see (**14**) (Fig. 2) for crystallographic numbering] was investigated. It crystallized from *n*-hexane as orthorhombic crystals in space group $P2_12_12_1$ but this time with four molecules per unit cell. The X-ray structure was solved by direct methods using diffractometer data to $R = 5.36\%$ over 1716 observed reflections. The resulting structure for α-ketol (**14**) is shown in Fig. 4.

Bond lengths for β-CBD and the α-ketol are shown in Table 4, together with their standard deviations. Except for the obvious differences between the allylic alcchol system of β-CBD and the conjugated ketone system of the α-ketol, and having regard for the higher standard

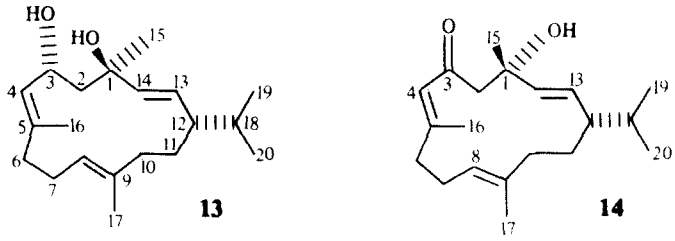

Fig. 2. Crystallographic numbering of β-cembrenediol (**13**) and α-cembreneketone (**14**) for X-ray purposes.

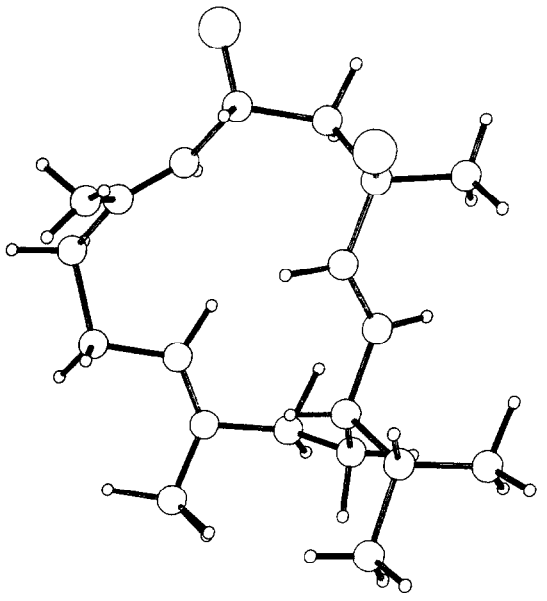

Fig. 3. Structure of β-cembrenediol (**13**).

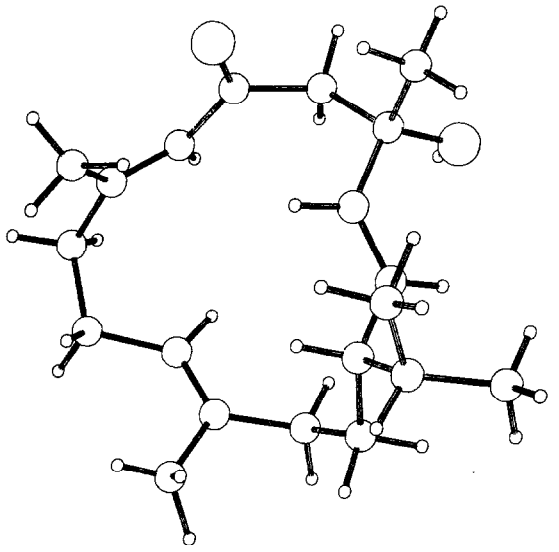

Fig. 4. Structure of α-cembreneketol (**14**).

deviations of β-CBD (particularly the disordered regions), other bond lengths in the two structures are very similar. Bond angles for β-CBD and the α-ketol are listed in Table 5. These again adopt expected values and, with the reservations mentioned, are similar within their standard deviations, as between the compounds. It is of interest that the bond angles at unmethylated sp^2 hybridized carbon atoms in the cembrene ring (crystallographic numbering C-4, C-8, C-13, C-14) are all enlarged by similar amounts in the two structures. It is also noted that the C-14–C-1–β-site bond angle is greater than the C-14–C-1–α-site bond angle in each structure.

The torsion angles for each structure around the 14-membered ring and involving the substituents at C-1 and C-3 are listed in Table 6. These are very similar, confirming similar ring conformations. However, the change in

Table 4. Bond lengths in Å with standard deviations in parentheses

		α-ketol (**14**)	β-CBD (**13**)
C1	C2	1.544 (4)	1.54 (2)
C1	C14	1.519 (4)	1.50 (1)
C1	C15	1.525 (4)	1.53 (2)
C1	O1	1.426 (3)	1.42 (1)
C2	C3	1.513 (4)	1.52 (1)
C3	C4	1.457 (4)	1.49 (2)
C3	O3	1.226 (4)	1.45 (1)
C4	C5	1.336 (4)	1.32 (2)
C5	C6	1.495 (4)	1.52 (2)
C5	C16	1.499 (5)	1.50 (2)
C6	C7	1.531 (5)	1.65 (3)
C7	C8	1.506 (5)	1.49 (3)
C8	C9	1.330 (4)	1.27 (3)
C9	C10	1.507 (5)	1.45 (9)
C9	C17	1.479 (5)	1.41 (5)
C10	C11	1.525 (6)	1.37 (5)
C11	C12	1.538 (6)	1.46 (3)
C12	C13	1.498 (4)	1.51 (2)
C12	C18	1.556 (5)	1.60 (3)
C13	C14	1.321 (4)	1.31 (1)
C18	C19	1.508 (7)	1.56 (4)
C18	C20	1.509 (7)	1.42 (5)

stereochemistry at C-1 from α- to β- does cause a change in ring conformation at this site. All torsion angles about the C-1–C-2 bond are changed +20° from the α-ketol (**14**) to β-CBD (**13**), while all torsion angles about the C-1–C-14 bond are twisted −18° between the same two structures. The change in hybridization at C-3 is accompanied by a +19° twist about the C-2–C-3 bond and a corresponding −15° twist about the C-3–C-4 bond. The differences in the C-8, C-9, C-10 portion of the ring are associated with high standard deviations in these angles for β-CBD and are probably not significant. Comparison of the ring shapes with similar hydroxy-cembrenes would be interesting but atomic co-ordinate data are not available [14, 15].

Intermolecular hydrogen bonds were located in both structures. In the α-ketol there is one such bond O–1–O-3 $(1-x, \frac{1}{2}+y, \frac{1}{2}-z)$ 2.90 Å, while in β-CBD there are two bonds O-1–O-3 $(1-y, x-y-1, \frac{1}{3}+z)$ 2.75 Å and O-3–O-3 $(x-y, -y, 5/3-z)$ 2.65 Å. For the α-ketol the hydrogen atom was located in a difference map and showed an O–H–O bond angle of 149°.

β-CBD in solution in dry (refluxed 24 hr P_2O_5 under N_2) carbon tetrachloride (3.32 mM) showed no evidence of intramolecular hydrogen bonding when examined by Fourier Transform IR spectroscopy (only a free secondary OH, 3612 cm^{-1}). By contrast, α-CBD (3.55 mM in dry CCl_4) showed a free and an intramolecularly bonded hydroxyl [3611 and 3516 cm^{-1}, $\Delta\nu$(OH)95 cm^{-1}]. Substitution into Kuhn's empirical relationship [16]:

$$\Delta\nu(\mathrm{OH}) = \frac{250}{d_{\mathrm{H}\ldots\mathrm{O}}} - 74\ \mathrm{cm}^{-1}$$

gives 1.48 Å as the separation between the donor hydrogen and the acceptor oxygen. Such a hydrogen bond [17] imposes substantial conformational changes on the

Table 5. Bond angles in degrees with standard deviations in parentheses

			α-ketol (14)	β-CBD (13)
C2	C1	C14	111.5 (2)	114 (1)
C2	C1	C15	110.9 (3)	108 (1)
C2	C1	O1	109.0 (2)	109 (1)
C14	C1	C15	108.4 (3)	113 (1)
C14	C1	O1	111.4 (2)	107 (1)
C15	C1	O1	105.5 (2)	107 (1)
C1	C2	C3	114.0 (2)	119 (1)
C2	C3	C4	115.6 (2)	114 (1)
C2	C3	O3	120.2 (3)	106 (1)
C4	C3	O3	124.2 (3)	106 (1)
C3	C4	C5	128.5 (3)	128 (1)
C4	C5	C6	120.7 (3)	121 (1)
C4	C5	C16	122.6 (3)	125 (1)
C6	C5	C16	116.6 (3)	114 (1)
C5	C6	C7	112.0 (3)	111 (1)
C6	C7	C8	112.0 (3)	110 (1)
C7	C8	C9	127.1 (3)	122 (4)
C8	C9	C10	120.5 (3)	116 (5)
C8	C9	C17	122.8 (3)	133 (6)
C10	C9	C17	116.7 (3)	109 (3)
C9	C10	C11	115.1 (3)	135 (4)
C10	C11	C12	113.9 (3)	109 (2)
C11	C12	C13	112.0 (3)	113 (1)
C11	C12	C18	111.0 (3)	110 (2)
C13	C12	C18	111.9 (3)	107 (1)
C12	C13	C14	124.0 (3)	126 (1)
C1	C14	C13	127.0 (3)	128 (1)
C12	C18	C19	113.8 (4)	112 (2)
C12	C18	C20	110.9 (3)	118 (2)
C19	C18	C20	110.1 (5)	107 (2)

Table 6. Selected torsion angles

				α-ketol (14)	β-CBD (13)
C14	C1	C2	C3	+41	+59
C1	C2	C3	C4	−108	−89
C2	C3	C4	C5	+152	+137
C3	C4	C5	C6	−172	+177
C4	C5	C6	C7	+105	+99
C5	C6	C7	C8	−62	−66
C6	C7	C8	C9	+162	−168
C7	C8	C9	C10	+178	−169
C8	C9	C10	C11	+125	+99
C9	C10	C11	C12	−58	−46
C10	C11	C12	C13	−65	−82
C11	C12	C13	C14	+135	+137
C12	C13	C14	C1	+173	+173
C13	C14	C1	C2	+131	+114
O1	C1	C2	C3	+164	−60
O1	C1	C14	C13	+9	−126
C15	C1	C2	C3	−80	−175
C15	C1	C14	C13	−107	−9
O3	C3	C2	C1	+70	+155
O3	C3	C4	C5	−26	−106

α-isomer and these are doubtless to be associated with the considerable differences which exist between the α- and β-CBD's, epimeric at C-4.

In Tables 7 and 8 we also record 2D $^{13}C/^{1}H$ correlation spectra for both the α-and β-cembrenediols. Our ^{13}C spectral data agree well with those recorded by Wahlberg and her colleagues [18], but there is minor variation from an earlier set of assignments [13].

EXPERIMENTAL

[U-^{14}C]-*Labelled α- and β-cembrenediols from photosynthesis in tobacco using* $^{14}CO_2$. A young tobacco plant, 18″ high, was sealed within a large transparent plastic bag connected to apparatus for generating and pumping $^{14}CO_2$ from $Ba^{14}CO_3$ (150 mg), 5 mCi) treated with 2 M HCl (3 ml). Towards the end of the generation period the generating flask was warmed and any remaining $^{14}CO_2$ pumped into the bag which was then sealed and left in sunshine for 6 hr. The bag was opened, any excess $^{14}CO_2$ dispersed, and the plant was removed to a warm greenhouse and allowed to grow normally for 8 days. Surface lipids were removed by dipping the leaves in CH_2Cl_2. Evapn gave 0.69 g material of total activity 50.45 μCi. Leaves and stems were freeze-dried and ground and gave 7.98 g material.

Tobacco leaf surface extract (34.8 μCi) was partitioned between hexane and MeOH–H_2O (9:1) giving a non-polar fraction (8.5 μCi) and washings (0.9 μCi) and a polar fraction (25.2 μCi), 35% by weight of the original leaf surface extract. The latter was dissolved in MeOH (5 ml) and injected via a 500 μl sample loop onto a reversed phase C_{18}-HPLC column eluting with MeOH–H_2O (3:1). Three fractions were collected: (1) material more polar than α-CBD (12.8 μCi), α-CBD (9.65 μCi) (3) β-CBD (3.7 μCi) and (4) residues (0.25 μCi).

After dilution with authentic 'cold' material (92 mg), [U-^{14}C]-β-CBD was crystallized from hexane, cooling to -40°, to constant specific activity, yielding a 75% recovery as colourless needles. [U-^{14}C]-α-CBD diluted with cold material (103 mg), was similarly crystallized from *n*-pentane, cooling to −40° for several days. This gave pure α–material (28% recovery) as a white powder. Concn of the mother liquors yielded a second crop (48% recovery).

Oxidation of α-cembrenediol to ketone (**9**) CrO_3 (100 mg, 1.0 mmol) was added to pyridine (0.24 ml, 3.0 mmol) in CH_2Cl_2 (20 ml) at 0°. After stirring at 0° (30 min), α-cembrenediol (100 mg, 0.327 mmol) in CH_2Cl_2 (1 ml) was added and the mixture stirred (2 hr, 0°). Extraction and chromatography on silica (elutant ether hexane 1:2) gave the enone (**9**) (80 mg, 80%). It crystallized from *n*-pentane in needles mp 78–79° (Lit. [4] mp 77–78°) (Found: C, 79.0; H, 10.9; M^+ 304. $C_{20}H_{32}O_2$ requires C, 78.9; H, 10.6%, M 304). ^{1}H NMR ($CDCl_3$) δ:6.05 (1H, *s*, 7-H), 5.55–5.35 (2H, *m*, 2-H and 3-H), 4.95 (1H, *m*, 11-H), 2.73 (1H, *d*, J = 12 Hz, CHH′.CCO) 2.52 (1H, *d*, J = 12 Hz, CHH′C=O), 2.4–1.2 (11H, *m*), 2.08 (3H, *d*, J = 1.2 Hz, 19-H), 1.56 (3H, *s*, 20-H), 1.34 (3H, *s*, 18-H), 0.82 (3H, *d*, J = 8.8 Hz, Me. CHMe), 0.80 (3H, *d*, J=8.8, MeCH.Me): ν_{max}($CHCl_3$) 1680 cm^{-1} (.C=C.C=O).

Shaking α-CBD (90 mg) for 40 hr with active MnO_2 (600 mg) in CH_2Cl_2 (10 ml) in N_2 also gave ketone (**9**) (95% yield).

Reduction of α-Enone (**9**) *with sodium borotritide.* Cerium trichloride nonahydrate (18 mg, 0.049 mmol) [12] was added to a solution of α-enone (**9**) (15 mg, 0.049 mmol) in MeOH (4 ml) and set aside for 5 min. Sodium borotritide (20 mCi, 3 mg) in MeOH (2 ml) was added: TLC after 10 min indicated consumption of the starting material. After evapn the residue was taken into water and extracted x3 with ether. Drying and evapn gave a mixture of two isomeric diols which were separated by HPLC

Table 7. 400 MHz ^{1}H–100 MHz ^{13}C 2D correlation NMR spectrum for α-cembrenediol (**1**)

δ^{13}C/ppm	δ^1H/ppm	Multiplicity	J(Hz)	Assignment*
15.08	1.52	*s*	—	C-20,Me
16.15	1.67	*d*	1.2	C-19,Me
19.42	0.79	*d*	6.8	C-17, Me
20.74	0.82	*d*	6.7	C-16, Me
23.39	2.18	*m*	1.2, 4.9, 6.9	C-10, CH_2
28.02	1.32	*m*	1.7, 4.2, 9.7	C-14, CH_b
28.02	1.61	*m*	3.5, 6.9	C-14, CH_a
30.19	1.35	*s*	—	C-18, Me
33.08	1.50	*dq*	1.4, 6.9	C-15, CH
36.88	1.98	*dm*	3.6, 8.3	C-13, CH_2
38.94	2.13	*m*	1.2, 4.9	C-9, CH_2
46.48	1.59	*dm*	1.4, 3.4	C-1, CH
52.24	2.03	*m*	2.2, 11.1	C-5, CH_2
66.42	4.48	*dt*	2.2, 8.9	C-6, CH
72.55	—	—	—	C-4, C
124.49	5.03	*dt*	1.2, 6.9	C-11, CH
127.67	5.33	*dd*	3.6, 15.4	C-2, CH
130.66	5.31	*dm*	2.2, 3.6, 8.9	C-7, CH
133.50	—	—	—	C-12, C
136.96	—	—	—	C-8, C
137.62	5.32	*dd*	1.4, 15.3	C-3, CH

*Chemical numbering.

Table 8. 400 MHz ^{1}H–100 MHz ^{13}C 2D correlation NMR spectrum for β-cembrenediol (**2**)

δ^{13}C/ppm	δ^1H/ppm	Multiplicity	J(Hz)	Assignment*
15.03	1.50	*s*	—	C-20, Me
15.98	1.70	*s*	—	C-19, Me
19.44	0.79	*d*	6.7	C-17, Me
20.66	0.82	*d*	6.7	C-16, Me
23.14	2.13	*dm*	5.1, 13.2	C-10, CH_2
27.75	1.30	*dd*	3.7 8.1	C-14, CH_b
27.75	1.56	*t*	3.4	C-14, CH_a
28.83	1.40	*s*	—	C-18, Me
33.00	1.47	*dd*	3.7, 7.3	C-15, CH
36.57	1.90	*dd*	4.4, 11.7	C-13, CH_a
36.57	2.01	*m*	2.2	C-13, CH_b
38.91	2.15	*dm*	5.1, 11.0	C-9, CH_2
46.34	1.54	*dd*	3.2, 8.1	C-1, CH
52.56	1.86	*dd*	8.8, 13.2	C-5, CH_a
52.54	2.03	*dd*	1.3, 14.2	C-5, CH_b
64.55	4.81	*dt*	1.4, 9.2	C-6, CH
71.56	—	—	—	C-4, C
124.50	4.99	*t*	4.5	C-11, CH
130.48	5.19	*d*	9.1	C-2, CH
131.50	5.24	*dt*	1.1, 10.0	C-7, CH
133.13	—	—	—	C-12, C
136.22	5.39	*d*	15.6	C-3, CH
136.57	—	—	—	C-8, C

*Chemical numbering.

on silica, eluting with ether–hexane (2:1). As estimated by peak area, the proportions were 4α-,6α-diol (**1**) 75% and 4α-,6β-diol (**11**) 25%, the total yield being 70%. The 4α-,6α-cembrenediol ([6β-^{3}H]-α CBD) (5.3 mg) had a radioactivity of 1.79×10^8 dpm/mg, *m/z* 288 (M-H_2O, 288), ^{1}H NMR ($CDCl_3$) δ:5.37–5.30 (3H, *m*, 2-3-and 7-H), 5.04 (1H, *t*, *J* 3.6 Hz, 11-H), 4.49 (1H, *td*, *J* = 8.2 and 3 Hz, 6-H), 2.3–1.2 (14H, *m*), 1.67 (3H, *d*, *J* = 1.2 Hz, 19-H), 1.52 (3H, *s*, 20-H), 1.35 (3H, *s*, 18-H), 0.82 (3H, *d*, *J* = 7.8 Hz, Me.CHMe), 0.80 (3H, *d*, *J* = 7.8 Hz, MeCH.Me).

The 4α-,6β-cembrenediol (5.3 mg) had a radioactivity of 4.68 $\times 10^3$ dpm/mg; *m/z* 288 (M − H_2O, 288), ^{1}H NMR ($CDCl_3$) δ:5.83 (1H, *d*, *J* = 15.7 Hz, 3-H), 5.38 (1H, *q*, *J* = 15.4 and 8.4 Hz, 2-H), 5.33 (1H, *d*, *J* = 8.6 Hz, 7-H), 4.91 (1H, *m*, 11-H), 4.72 (1H, *d*, *J* = 9.5 and 3.7 Hz, 6-H), 2.4–1.2 (14H, *m*), 1.74 (3H, *d*, *J* = 5 Hz, 19-H), 1.55 (3H, *s*, 20-H), 1.36 (3H, *s*, 18-H), 0.84 (3H, *d*, *J* = 9.5 Hz, Me.CHMe), 0.81 (3H, *d*, *J* = 9.5 Hz, MeCH.Me).

A similar reaction using unlabelled α-ketol (27.2 mg), cerium trichloride heptahydrate (38.5 mg) and $NaBH_4$ (5.9 mg) in MeOH (3 ml) gave 4α,6α- diol (12.6 mg, 46%) and 4α,6β diol (4.5 mg, 16%) after isolation by HPLC. A sodium borodeuteride reduction of the α-enone in which the cerium reagent was omitted gave the expected product lacking the proton signal at δ4.48 but it was contaminated with 1,4-reduction product. Similar results were obtained with the β-enone.

Oxidation of β-cembrenediol to ketone (**10**). Oxidation of β-cembrenediol (100 mg, 0.327 mmol) in a manner analogous to that used for the α- (above), followed by chromatography, gave the enone (**10**) an oil (49 mg, 49%), ^{1}H NMR δ($CDCl_3$): 6.09 (1H, *s*, 7-H), 5.4–5.2 (2H, *m*, 2- and 3-H), 4.95 (1H, *m*, 11-H), 2.77 (1H, *d*, *J* = 12.6 Hz, HH'C=O), 2.47, (1H, *d*, *J* = 12.6 Hz, HH'C=O), 2.4–1.2 (11H, *m*), 2.05 (3H, *d*, *J* = 0.9 Hz, 19-H), 1.53 (3H, *s*, 20-H), 1.36 (3H, *s*, 18-H), 0.82 (3H, *d*, *J* = 8.5 Hz, Me.CHMe), 0.80 (3H, *d*, *J* = 8.5 Hz, MeCH.Me). The active MnO_2 procedure (above) gave ketone (**10**) in 91% yield.

Reduction of β-enone (**10**) *with sodium borohydride.* Cerium trichloride nonahydrate (12 mg, 0.033 mmol) was added to the enone (**10**) (10 mg, 0.033 mmol) in MeOH (4 ml) and kept for 5 min. $NaBH_4$ (1.8 mg, 0.049 mmol) was introduced and after 10 min worked up as for the α-case (above). TLC indicated a 4β,6β (**12**) and 4β,6α (**2**) mixture (> 19:1). The major component, 4β,6β-cembrenediol (**12**), an oil, was isolated by chromatography on silica (8.8 mg, 88%) and had $[M - H_2O]^+$ 288.2447 ($C_{20}H_{34}O_2 - H_2O$ requires 288.2453). It had ^{1}H NMR ($CDCl_3$) δ:6.01 (1H, *d*, *J* = 15.7 Hz, 3-H), 5.49 (1H, *d*, *J* = 9.3 Hz, 7-H), 5.25 (1H, *q*, *J* = 15.7, 8.7 Hz, 2-H), 4.37 (1H, *d*, *J* = 9.4 Hz, 11-H), 4.74 (1H, *m*, *J* = 9.5, 7.0 and 2.5 Hz, 6-H), 2.4–1.2 (11H, *m*), 1.64 (3H, *s*, 19-H), 1.54 (3H, *s*, 20-H), 1.35 (3H, *s*, 18-H), 0.86 (3H, *d*, *J* = 7.0 Hz, Me.CHMe) 0.84 (3H, *d*, *J* = 7.0 Hz, MeCH.Me). A second reaction using β-ketol (30.2 mg), cerium trichloride heptahydrate (39.2 mg) and $NaBH_4$ (5.9 mg) in MeOH (3 ml) gave a 4β-6α ratio of 82:1 by HPLC. Isolated yields were 4β,6β (22.2 mg, 73%), 4β,6α (< 1 mg).

Single crystal X-Ray data

α-Cembrene 1,3-diol $C_{20}H_{34}O_2$, M = 306.472, orthorhombic, *a* = 19.294 (6), *b* = 28.342(5) and *c* = 32.678 (7) Å, *U* = 17869.91 Å^3, *z* = 36, D_c = 1.03 g cm^{-3}, space group $P2_12_12_1$.

α-Cembrene-1-ol-3-one (**14**). $C_{20}H_{32}O_2$, M = 304.456, orthorhombic, *a* = 10.015 (1), *b* = 10.922 (1) and *c* = 17.977 (1) Å, *U* = 1966.32 Å^3, *z* = 4, D_c = 1.03 g cm^{-3}, space group $P2_12_12_1$. *F*(000) = 672. CuK_α radiation λ = 1.54178 Å, μ = 5.02 cm^{-1}, crystal size 0.6 × 0.4 × 0.4 mm.

β-Cembrene-1,3-diol (**13**): $C_{20}H_{34}O_2$, M = 306.472, Trigonal, *a* = 16.979 (2) and *c* = 11.825 (1) Å, U = 2952.27 Å^3, *z* = 6, D_c = 1.03 g cm^{-3}, space group $P3_221$ *F*(000) = 1020, CuK_α radiation λ = 1.54178 Å, μ = 5.02 cm^{-1}, crystal size 0.65 × 0.4 × 0.1 mm.

Intensity measurement of (**14**) and (**13**) was performed on an Enraf-Nonius CAD4 diffractometer. For (**14**) an ω/2θ scan was used for $1° \leqslant \theta \leqslant 76°$. A total of 2346 independent reflections was measured of which 1716 had $I \geqslant 3\sigma(I)$ and were considered observed and used in the subsequent refinement. For (**13**) an ω/θ scan was used for $1° \leqslant \theta \leqslant 55°$. A total of 1634 independent reflections was measured of which only 949 had $I \geqslant 2\sigma(I)$ and were considered observed and used in the subsequent refinement. Both sets of data were corrected for Lorentz and polarization factors but not absorption. Data reduction and subsequent crystallographic calculations were performed using the CRYSTALS [19] system of programs.

Both structures were solved by direct methods using the MULTAN [20] program. In each case 19 atomic positions were located in an E map and the remaining 3 positions were obtained from a subsequent difference Fourier synthesis. Full matrix least squares refinement of atomic positions, initially isotropically and subsequently anisotropically, was used. The disorder in two regions of structure (**13**) caused some unusual bond lengths and angles to be seen in these regions and attempts were made to improve the refinement by applying constraints to these bond lengths and angles. As these caused no great improvement the constraints were subsequently removed. Difference maps showed some hydrogen atom positions so these positions were calculated from geometrical considerations and included in the structure factor calculations but without refinement. For structure (**14**) the OH hydrogen was located in a difference map and included, whereas those in (**13**) were not found. A weighting scheme based upon a Chebyshev polynomial was used in the final cycles of refinement. Final R values for (**14**) were 5.36% with R_w 6.77%. The corresponding values for (**13**) were 9.66% and R_w 13.0%. Final difference maps showed no features > 0.21 *e* Å^{-3} for (**14**) and > 0.35 *e* Å^{-3} for (**13**). Final atomic coordinates are listed in Table 9 for (**14**) and Table 10 for

Table 9. Fractional atomic co-ordinates for α-cembren-1-ol-3-one (**14**)

Atom	*x/a*	*y/b*	*z/c*
C(1)	0.3852 (3)	0.4733 (2)	0.2912 (2)
C(2)	0.4680 (3)	0.4958 (3)	0.2201 (2)
C(3)	0.5958 (3)	0.4222 (3)	0.2163 (1)
C(4)	0.7186 (3)	0.4917 (3)	0.2266 (2)
C(5)	0.8357 (3)	0.4532 (3)	0.2536 (2)
C(6)	0.9458 3)	0.5420 (4)	0.2690 (2)
C(7)	0.9622 (3)	0.5665 (3)	0.3524 (2)
C(8)	0.8393 (3)	0.6238 (3)	0.3857 (2)
C(9)	0.8070 (3)	0.6293 (3)	0.4574 (2)
C(10)	0.6811 (4)	0.6932 (4)	0.4819 (2)
C(11)	0.5871 (4)	0.6158 (4)	0.5293 (2)
C(12)	0.5411 (4)	0.4969 (3)	0.4914 (2)
C(13)	0.4586 (3)	0.5213 (3)	0.4235 (2)
C(14)	0.4739 (3)	0.4635 (2)	0.3595 (2)
C(15)	0.3040 (4)	0.3557 (3)	0.2847 (3)
C(16)	0.8620 (5)	0.3219 (4)	0.2728 (3)
C(17)	0.8926 (5)	0.5780 (5)	0.5167 (2)
C(18)	0.4674 (5)	0.4120 (4)	0.5475 (2)
C(19)	0.3342 (6)	0.4611 (6)	0.5728 (3)
C(20)	0.4509 (8)	0.2849 (5)	0.5160 (3)
O(1)	0.2896 (2)	0.5691 (2)	0.2990 (1)
O(3)	0.5917 (3)	0.3111 (2)	0.2071 (1)

Table 10. Fractional atomic co-ordinates for β-cembrene-1,3-diol (**13**)

Atom	x/a	y/b	z/c
C(1)	0.8822 (7)	0.1313 (7)	1.0192 (10)
C(2)	0.9233 (8)	0.1049 (9)	0.9196 (9)
C(3)	1.0259 (7)	0.1521 (8)	0.9061 (10)
C(4)	1.0651 (9)	0.2366 (8)	0.8370 (11)
C(5)	1.1394 (10)	0.3149 (11)	0.8568 (12)
C(6)	1.1719 (12)	0.3937 (12)	0.7742 (16)
C(7)	1.1415 (23)	0.4671 (10)	0.8161 (18)
C(8)	1.0405 (21)	0.4238 (16)	0.8123 (20)
C(9)	1.0011 (41)	0.4704 (33)	0.8220 (20)
C(10)	0.9034 (43)	0.4190 (45)	0.8386 (22)
C(11)	0.8472 (17)	0.3959 (12)	0.9302 (41)
C(12)	0.8822 (11)	0.3630 (9)	1.0198 (14)
C(13)	0.8551 (9)	0.2639 (9)	1.0056 (11)
C(14)	0.9080 (7)	0.2295 (8)	1.0204 (9)
C(15)	0.7789 (9)	0.0670 (9)	1.0173 (14)
C(16)	1.1938 (11)	0.3379 (12)	0.9639 (16)
C(17)	1.0331 (37)	0.5631 (27)	0.8455 (28)
C(18)	0.8450 (12)	0.3728 (14)	1.1403 (30)
C(19)	0.8725 (24)	0.4733 (24)	1.1661 (32)
C(20)	0.7504 (38)	0.3185 (27)	1.1627 (22)
O(1)	0.9140 (7)	0.1141 (6)	1.1220 (7)
O(3)	1.0448 (7)	0.0896 (6)	0.8442 (10)

(**13**). Hydrogen positions and temperature factors are available on request from the Cambridge Crystallographic Data Centre.

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