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BIOSYNTHETIC PRECURSORS FOR α- AND β-CEMBRENEDIOL FORMATION IN TOBACCO

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; α- and β-cembrenediols; geranylgeraniol; casbene; cembrene; isotopic labelling; biosynthesis.

Abstract—After survey of the α- and β-cembrenediol (CBD) content of different plant organs of tobacco, calyces were selected for tracer administration. (*R/S*)-[2-^{14}C]-Mevalonic acid dibenzylethylenediamine salt gave incorporations of 0.10% into α-CBD and 0.03% into β-CBD. Good incorporations (α-CBD 2.30%, β-CBD 0.93%) of sodium [1-^{14}C] acetate were found. *all*-(*E*)-[2-^{14}C]-Geranylgeraniol gave incorporations of 1.49 and 1.53% into the cembrenediols in two experiments, though radiochemical distribution among α- and β- forms was variable. Using the Kato cyclization method, a sample of (*R/S*)-[3-^{14}C]-cembrene was prepared and was incorporated into the cembrenediols (0.58 and 0.42% overall, with variable distribution among α- and β-isomers). Methods for making (*R/S*)-[2-^{2}H], [15-^{2}H] and *ent*-(1*R*)-[20-^{3}H] cembrene are described. The possible involvement of *ent*-(1*R*,3*S*)-casbene in the formation of (1*S*)-cembrene is discussed.

INTRODUCTION

The leaf trichomes of the commercial tobacco plant (*Nicotiana tabacum*), a hybrid of *N. sylvestris* (male) and *N. tomentosiformis* (female), secrete a cembrenoid-containing gum onto the leaf surface [1, 2]. This gum has plant growth inhibiting properties, attributed to the cembrenoids it contains, suppressing the development of axillary shoots [3]. It also has insecticidal properties [4, 5]. The major cembrenoid† components of the gum are (1*S*,4*S*,6*R*,2*E*,7*E*,11*E*)-4,6-*dihydroxycembra*-2,7,11-*triene* (**1**) (α-cembrenediol or α-CBD) [6, 7] and its (4*R*)-epimer (**2**) (β-cembrenediol or β-CBD) [3, 6, 8]; also occurring with them are a number of minor relatives believed to be formed from these two structures by further metabolic activity [9–12]. The two major cembrenediols are recognised as being of considerable interest to the tobacco industry since degradation products produced on curing and smoking are significant contributors to tobacco flavours, particularly those of the Virginia and Burley types [6, 13, 14]. Recently the two cembrenoids have also been recognised as potent inhibitors of the early antigen of the Epstein-Bar virus which can be induced by 12-*O*-tetradecanoylphorbol-13-acetate [15]. This evidence of anti-tumour-promoting effects has stimulated further interest in the α- and β-CBDs. There is little definite information on the biosynthesis of these compounds [2, 16] and the present work has been initiated to obtain basic information on the processes involved, using isotopically labelled precursors.

† In the earlier literature nomenclature based on the synonyms 'duvane' and 'thunbergane' was used for 'cembrane' which is now the widely accepted stem name with the numbering as in (**1**).

RESULTS AND DISCUSSION

Initially, tobacco leaves [17] separated from the plant and dipped into a solution of radioactive precursor were tried as an administration system, but experiments with sodium [1-^{14}C]-acetate gave low incorporations into the cembrenediols. A series of experiments to evaluate the α- and β-cembrenediol content associated with different tobacco plant organs was therefore carried out (Table 1). These suggested that tobacco calyces, rich in trichomes, were suitable, having a high diol/fresh weight ratio and showing active turnover. Using excised calyces from mature tobacco flowers, standing them in covered petri dishes containing a solution of sodium [1-^{14}C] acetate for 72 hr with illumination from a 40 W lamp, a total incorporation of 3.23% into the cembrenoid mixture was found. The cembrenoids were separated and purified by C_{18}-reversed phase HPLC, then being diluted with authentic cold material and crystallized five times to constant count. The individual incorporations into α-CBD were 2.30% and β-CBD 0.93%. Similar, though shorter (48 hr), feeding of (*R/S*)-[12-^{14}C]-mevalonic acid as its dibenzylethylenediamine salt, along with a little sucrose, gave a total incorporation of 0.13% into the CBDs, 0.1% into α- and 0.03% into β-. The incorporation of mevalonic lactone is modest, but poor incorporations are sometimes found in terpenic systems and often ascribed to 'compartmentation'. However, they gave adequate encouragement now to attempt experiments with larger candidate precursors and we turned our attention to geranylgeraniol (**3**).

For labelling of the latter in the 2-position, *all*-(*E*)-farnesylacetone (**4**) was required. This was made from a commercially available mixture of farnesol isomers which was separated into its four geometrical isomers by preparative C_{18}-reversed phase HPLC using a mobile

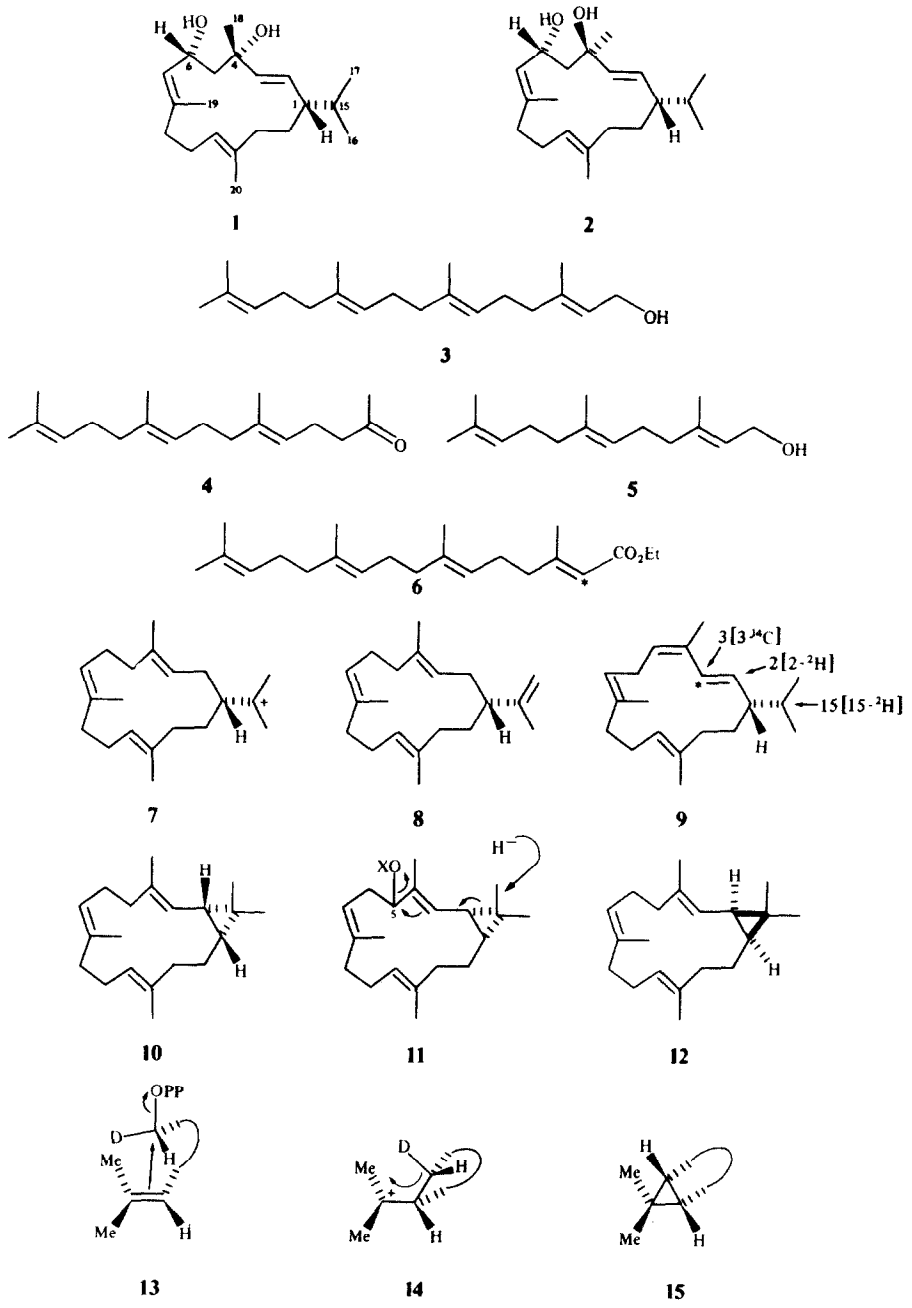


Table 1. The α- and β-cembrene-4,6-diol content of *N. tabacum* organs

Plant organ	No. of organs used	Fresh wt of sample (g)	Wt of α-(1)+β-(2) diols (mg)	% of α-(1)+β-(2) diols*	Ratio of α-(1)−β-(2) diols
Young upper leaves	3	50.1	73	0.15	43:57
Old lower leaves	3	47.1	20	0.04	39:61
Immature petals	24	3.7	18	0.49	76:24
Mature petals	49	10.5	61	0.58	75:25
Mature calyces	68	3.0	70	2.33	76:24
'Fertilized' calyces	45	2.5	63	2.52	60:40

*In fresh material.

phase of methanol–water (3:2) containing 12 mmol/l of silver nitrate [18]. An excellent separation of the four stereoisomers was attained, *all*-(*E*)-material (**5**) (first eluted) being isolated in 43% yield with a purity of >95% as determined by GC (Carbowax 20M, 50 m fused silica column). Conversion into the bromide and acetoacetic ester synthesis using the literature procedure [19] gave *all*-(*E*)-farnesylacetone. The latter was treated with the anion (sodium hydride) of diethyl [2-^{14}C]-carboethoxymethyl phosphonate made by Arbusov reaction between ethyl [2-^{14}C]-bromoacetate and triethyl phosphite. The C_{20}-product was freed from (2*Z*)-contaminant by plate chromatography and the *all*-(*E*)-isomer of ethyl geranylgeranoate isolated was reduced with di-isobutyl aluminium hydride at −78° to give *all*-(*E*)-geranylgeraniol (**3**) labelled at C-2. Administered to tobacco calyces, an incorporation of 1.49% into the mixture of α- and β-CBDs was obtained from the latter: a second experiment gave 1.53% incorporation (Table 2).

In the biosynthesis of the CBDs cyclization must occur using either (**3**) or a hydroxylated derivative. The former seems more likely, but then the nature of the initial cyclization product is uncertain. An obvious product of acid catalysed cyclization of geranylgeraniol is the carbonium ion (**7**) which leads to cembrene-A (neocembrene) (**8**). On the other hand, (1*S*)-cembrene (**9**) [20–22] itself looks a more attractive precursor of the CBDs and indeed there are reports of its detection in tobacco [16, 23]. (1*S*)-Cembrene itself might originate from *ent*-(1*R*,3*S*)-casbene (**10**) via 5-hydroxylation, solvolysis and reduction of the carbonium ion (cf. **11**). (1*S*,3R)-Casbene (**12**)* was discovered by West [24, 25] in germinated *Ricinus communis* seeds and its structure and stereochemistry derive from our earlier synthetic work [26]. There is a report of a small amount of casbene co-occurring with cembrene in *Sinularia conferta* [29], a soft coral, which tends to suggest that there may be biosynthetic relationships. The mechanism by which geranylgeraniol is converted into the cyclopropane casbene however remains unclear, though the stereochemistry of the process has been studied and a possible mechanism proposed (**13–15**) [27]. As a result of these considerations it appeared important first to establish if cembrene (**9**) really is a precursor of the CBDs (**1**) and (**2**) in tobacco.

This objective required cembrene labelled with deuterium, tritium or [^{14}C]. Geranylgeranoic acid was available [^{14}C]-labelled or 'cold' via the synthetic work above and a 'cold' specimen was also obtained from natural *all*-(*E*) geranylgeraniol isolated from *Bixa orellana* L. seeds. The latter was oxidized by manganese dioxide to the aldehyde and then to the acid using sodium cyanide and silver oxide in methanol at 0° [30]. Conversion of [2-^{14}C]-geranylgeranoic acid into its acid chloride using thionyl chloride and pyridine in benzene at 0°, followed by cyclization employing the procedure of Kato and his colleagues [tin (IV) chloride in dichloromethane at −78° with dilution] [19, 31, 32] gave (±)-[3-^{14}C]-15-chloro-3*E*,7*E*, 11*E*-trien-2-one (**16**).

Dehalogenation of the latter using tributyltin hydride in the presence of azobisisobutyronitrile gave the ketone (**17**) which was reduced with di-isobutyl aluminium hydride (DIBAL) to give (±)-[3-^{14}C]-mukulol (**18a**) and its C-2 epimer (**19a**). One of these predominated (9:1) (**18a**:**19a**). In a 'cold' experiment these diastereoisomers were separated chromatographically, but for the present purpose the mixture was dehydrated (thionyl chloride/pyridine/0°) to give (*R*/*S*)-[3-^{14}C]-cembrene (**9**) along with stereoisomeric contaminants which were removed by chromatography on silver nitrate impregnated silica. This labelled specimen was used for administration to tobacco calyces below, but first the labelling of cembrene with isotopic hydrogen was explored.

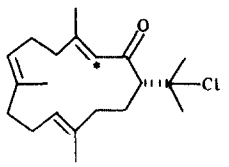
Two methods proceeded via 'cold' (**16**). Tributyltin magnesium bromide can be converted into the deuteride or tritide by treatment with D_2O or T_2O and use of this allowed us to place isotopic hydrogen at C-15 as in (**17b**), and hence by reduction (**18b**, **19b**) and dehydration as before, at C-15 in (*R*/*S*)-cembrene (**9**). Alternatively, isotopic hydrogen could be placed at C-2 in (*R*/*S*)-cembrene by carrying out reduction of unlabelled ketone (**17a**) with lithium aluminium deuteride. This gave (±)-[2-^{2}H]-mukulol and -epimukulol (cf. **20** and **21**) in a ratio of 37:13, but yields were reduced through accompanying 1,4-reduction. Separation, dehydration and purification as before, gave (*R*/*S*)-[2-^{2}H]-cembrene. As assessed by ^{1}H and ^{2}H NMR the deuterium labels of the (*R*/*S*)-[2-^{2}H]- and (*R*/*S*)-(15-^{2}H)-cembrenes were present at

*These 14-membered ring compounds occur naturally in both (1*R*) and (1*S*)-epimeric series. *ent*-(1*R*)-Cembrene occurs in the soft coral *Sinularia mayi* [28] and belongs to the Euphorbiaceae series, to which (1*S*,3*R*)-casbene belongs: the latter is the parent of an extensive range of natural compounds which includes lathyrol, bertiadionol, phorbol, ingenol etc. [26] *Pinus albicaulis* cembrene, like the tobacco compounds, belongs to the (1*S*)-series [13].

Table 2. Formation of α- and β-cembrene-4,6-diols from [2-^{14}C]-geranylgeraniol and (±)-[3-^{14}C]-cembrene by *N. tabacum* calyces

Precursor	Wt administered (mg)*	Total incorp. into CBDs (%)	α- and β-isomers in CBDs % α-	β-	Incorp. into CBD isomers % α-	β-
Exp. 1 *all*-(*E*)-[2-^{14}C]-Geranylgeraniol (**3**)	4.1†	1.49	65.8	34.2	1.3	0.19
Exp. 2 *all*-(*E*)-[2-^{14}C]-Geranylgeraniol (**3**)	4.1†	1.53	81.7	18.3	0.62	0.91
Exp. 3 (±)-[3-^{14}C]-Cembrene (**9**)	7.0‡	0.58	72.5	27.5	0.48	0.10
Exp. 4 (±)-[3-^{14}C]-Cembrene (**9**)	6.6§	0.42	73.0	27.0	0.23	0.19

*To 25 calyces from mature tobacco plants.
†112 495 dpm.
‡201 697 dpm.
§190 172 dpm.



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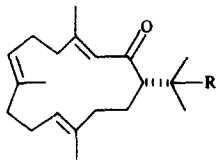
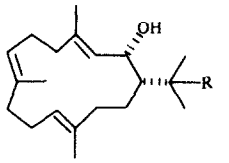


17a R = H
17b R = D



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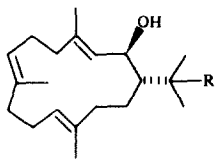
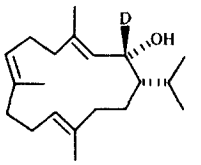


19a R = H
19b R = D



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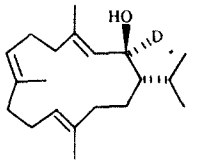

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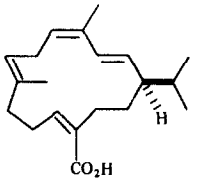

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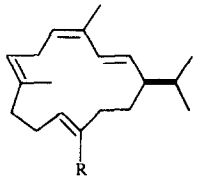
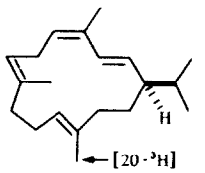


23a R = CO_2Me
23b R = CH_2OH
23c R = CH_2OMs
23d R = CHO
23e R = CH_2Cl
23f R = CH_2Br



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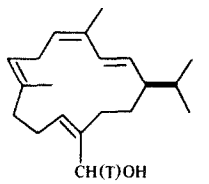

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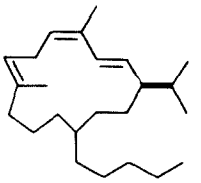

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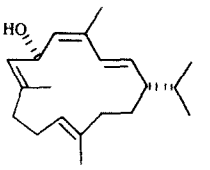

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>99% levels. Except for chirality and labelling, the synthetic cembrenes were spectroscopically identical with authentic (1*S*)-cembrene isolated from a suitable turpentine.

Chiral cembrene enantiomeric at C-1 (*ent*-cembrene) was also made with isotopic hydrogen at the C-20 methyl in the following way. Poilaneic acid (**22**) (1*R*,2*E*,4*Z*,7*E*,11*Z*-12-carboxy-1-isopropyl-4,8-dimethylcyclotetradecatetraene) is a natural product isolated from the Thai plant *Croton poilanei* Gagnep and it has indeed already been demonstrated by Sato *et al.* [33] in connection with structure work that it can be degraded to *ent*-cembrene. Treatment of its methyl ester (**23a**) with lithium aluminium hydride gave the alcohol (**23b**) which was mesylated (**23c**) and then reduced with lithium aluminium hydride to form *ent*-(1*R*)-cembrene (**24**). Except that in our hands the mesylation using mesyl chloride gave the chloride (**23e**) rather than the mesylate, the sequence could be repeated using lithium aluminium deuteride to give (**24**) deuterated at C-20. The lack of availability of lithium aluminium tritide however makes this sequence unattractive from a radiochemical point of view. Both sodium borodeuteride and sodium borotritide are readily available however, so alcohol (**23b**) was oxidised (chromium trioxide–pyridine) to aldehyde (**23d**) and the isotope was inserted at this point by reduction of the aldehyde with cerium trichloride–sodium borotritide reagent [34]. Conversion to the chloride (cf. **23e**) and reduction with lithium aluminium hydride then gave *ent*-cembrene (**24**) labelled at C-20 with one tritium atom. During experiments on the labelling at C-20, the bromide (**23f**) was prepared and treated with *n*-butyl lithium in the hope that lithiation might occur and isotopic hydrogen be introduced at C-20 on treatment with T_2O. Characterized by mass spectrometry and NMR however, the product was that from nucleophilic displacement by the *n*-butyl group (**26**). Whilst the availability of (*R*/*S*)-[2-^{2}H]- and -[15-^{2}H]-cembrenes and their tritiated equivalents should have general utility, (1*R*)-[20-^{3}H]-*ent*-cembrene and its deuterated equivalent may be of interest in distinguishing enzymic from non-enzymic processes in which the chirality at C-1 is critical to enzymic action in tobacco.

Administration of (*R*/*S*)-[3-^{14}C]-cembrene in water containing a little methoxyethanol and 'Tween 20' to *N. tabacum* calyces gave, in two experiments, incorporations into the cembrenediols of 0.58 and 0.42%. This is somewhat lower than for the putatively less close precursor geranylgeraniol (Table 2), but cembrene is more difficult to administer, is probably less easily transported, and is a (±)-compound, so no great significance is attached to this quantitative aspect. The ratios of the amounts of α- to β-CBDs present was in the range 65–85% 15–35% but the relative radiochemical incorporations into the two CBDs was more erratic and cannot be readily explained at this stage. Many variables however have not been controlled such as weather and other environmental factors, age of plants and age of calyces, time of administration etc., and these await more detailed studies.

Our results thus indicate a framework for the biosynthesis of the α- and β-cembrenediols, backed by some experimental evidence. The usual mevalonate pathway leads to geranylgeraniol which is an acceptable precursor. Steps leading via cyclization to cembrene seem best explained as proceeding through *ent*-casbene (**10**) rather than cembrene-A (**8**), the former being oxidised at C-5 and elimination proceding with reduction of the carbonium ion (cf. **11**). Labelled cembrene is shown to be a satisfactory precursor for the diols. It seems likely that stereospecific enzymic hydroxylation of cembrene at the allylic C-6 position precedes the hydration of the 4,5-double bond which is either non-stereospecific or is effected by two different hydrating enzymes. These views are

summarized in Fig. 1: experiments with labelled *ent*-casbene and labelled cembrene-A are now required to make further progress.

EXPERIMENTAL

α- and β-Cembrenediol content of tobacco plant organs. The plant material was extracted with $CHCl_3$ and the extract was evapd and partitioned between hexane and MeOH–H_2O (9:1). The MeOH–H_2O was evapd and the mixture of α- and β-CBDs was separated and estimated by analytical HPLC using an 8 mm, 10 μ particle Waters C_{18}-Rad Pak eluting with MeOH–H_2O 3:1 (Table 1).

Tobacco seeds (3.3 g, washed with hexane) were also examined for CBD content. After crushing and Soxhlet extraction for 13 hr with MeOH, the extract was partitioned between hexane and MeOH–H_2O (9:1). Evapn of the latter fraction and HPLC examination gave no indications of CBDs. Tests showed that using refractive index detection with the Rad Pak conditions mentioned above, the lower limit of detection for the CBDs was about 0.001 mg/injection (25 μl).

Isolation of α- and β-cembrenediols from tobacco calyces and tobacco leaves. Tobacco calyces (47) were washed for 30 sec with $CHCl_3$ (2 × 100 ml). Treatment as above by evapn to give a green gum, partition between hexane (60 ml) and MeOH–H_2O (9:1) (25 ml), and separation of the latter layer which was then washed with hexane (50 ml) and evapd, gave a gum. HPLC as above afforded α-cembrenediol (16.4 mg) and β-cembrenediol (4.2 mg).

For large scale (multigram) preparations leaf-surface extract from tobacco leaves (obtained by dipping in CH_2Cl_2) was used. The greenish waxy product was dissolved in hot Me_2CO and kept in a freezer to ppt. the bulk of the long-chain alkanes. After filtration the filtrate was concd and purified by preparative HPLC on a Waters 500 instrument using a C_{18}-reversed phase column and eluting with MeOH–H_2O (3:1). [An alternative treatment was partition of the leaf-surface extract between hexane and 90% MeOH containing 10% H_2O. The crude diols were recovered from the latter layer and purified by preparative HPLC.] The α-isomer eluted first and the first part of this peak was collected and the remainder recycled along with the β-peak. The procedure was repeated. The separated materials were concd on a rotary evaporator to remove most of the MeOH and then thoroughly extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried, filtered and concd. Both diols crystallize from hexane as fine needles, α-CBD mp 62–63° (Lit. [6] mp 65–66°), $[\alpha]^{20}$ +182° ($CHCl_3$; *c* 3.2 mg/ml) [Lit. [6] $[\alpha]^{25}$ +282° ($CHCl_3$)] and β-CBD mp 122–127° (Lit. [6] mp 127–127.5°), $[\alpha]^{20}$ +164°

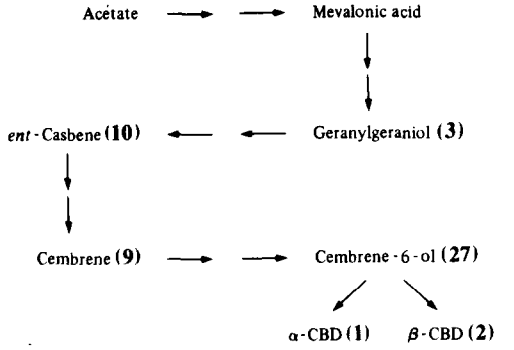

Fig. 1. A tentative biogenetic scheme for the major tobacco cembrenoids.

($CHCl_3$; *c* 1.7 mg/ml) [Lit. [6] $[\alpha]_D$ +162° ($CHCl_3$)]. β-CBD is considerably more stable to light and air than its α-epimer.

Administration of radioactive precursors to tobacco calyces. Calyces from mature tobacco plants (25–30) were removed and stood upright in a solution of radio-labelled substrate in 2-methoxyethanol (10 drops), Tween 20 (3 drops) and H_2O (4.2 ml). The calyces were set aside in covered petri-dishes for 48 hr at 28–30° under illumination. The calyces were removed and washed with $CHCl_3$ (2 × 200 ml, 1 × 50 ml) for 30 sec per wash, the washings were united and the solvent removed *in vacuo*. After partition between hexane and MeOH–H_2O (9:1), the aq.–alcoholic phase was washed with hexane (2 × 25 ml). The washings were rejected and the aq.–alcoholic solution was evapd to give a green gum which was purified by reversed-phase HPLC [8 mm, 10 μ particle C_{18}- Rad Pak for Waters Z-module eluting with MeOH–H_2O (3:1) at 2 ml/min] to give specimens of α- and β-CBDs. Each was diluted with authentic 'cold' material and crystallized to constant count–usually four or five crystallizations. α-CBD was crystallized from pentane, β-CBD from hexane. The above describes the administration method for [2-^{14}C]-geranylgeraniol and (±)-[3-^{14}C]-cembrene.

In the case of Na [1-^{14}C]-acetate (250 μCi), administration was in distilled H_2O (5 ml) containing 0.5 mmol/l of sucrose with illumination for 72 hr. (±)-[2-^{14}C]-Mevalonic acid DBED Salt (50 μCi) was administered in distilled water at 25° for 48 hr with illumination.

Ethyl all-(E)-[2^{14}*C*]-*geranylgeranoate* (**6**). Ethyl[2^{14}C]-bromoacetate (500 μCi) was diluted with cold material (312.2 mg, 1.37 mmol), triethyl phosphite (621 mg, 3.74 mmol) was added and the mixture refluxed in N_2 for 4 hr. After cooling, dry benzene (3 ml) and NaH (61.6 mg, 2.57 mmol) were added and the mixture was stirred at room temp. in N_2 until gas evolution ceased. *all-E*-Farnesylacetone (**4**) (405 mg., 1.54 mmol) in dry benzene (4 ml) was added and stirring in N_2 continued (20 hr). Work-up with saturated brine followed by chromatography on silica gel HF 254 (elutant 10% ether in hexane) gave ethyl *all-E* [2-^{14}C]-geranylgeranoate (**6**) (250 mg, 42%), R_f 0.73, ethyl (2*Z*,6*E*,10*E*)-[2^{14}C]-geranylgeranoate (29 mg, 5%), R_f 0.81 and unreacted farnesyl acetone (130 mg). A 'cold' run using farnesyl acetone (10 g) gave *all-(E)*-(6) (71%), $[M]^+$ 332.2658 (Calc. for $C_{22}H_{36}O_2$: M 332.2715), ν_{max} 1715 (C=O), 1666 and 1645 (.C=C.)cm^{-1}, and its (2*Z*) isomer (12%).

all-(E)-[2^{14}C]-*Geranylgeraniol* (**3**). Diisobutylaluminium hydride (0.1 M, 3.2 ml) in toluene–hexane was added dropwise to a stirred solution of ethyl *all-(E)*-[2-^{14}C]-geranylgeranoate (29.2 mg, 87.8 μmol) in dry hexane (3 ml) at −78° in N_2, stirring being continued for a further 2 hr at −78°. The reaction mixture was allowed to attain room temp. and then poured into ether–H_2O (3:1). Aq. NaOH (2 M) was added until all the ppt. had dissolved, and the phases were separated. The alkaline aqueous phase was extracted thoroughly with ether. Washing (saturated brine), drying (Na_2SO_4) and evapn gave a product which was purified by normal phase HPLC (8 mm, 10 μ Rad Pak eluting with 12.5% EtOAc in hexane) to give *all-(E)*-[2-^{14}C]-geranylgeraniol (**3**) (15.4 mg, 61%) R_f 0.14 (10% ether in hexane). The activity was 27.44 × 10^3 dpm/mg.

all-(E)-Geranylgeraniol had ^{1}H NMR spectrum δ($CDCl_3$): 5.42 (1H, *br d*, J_1=J_2, 5.8 Hz, J_3=1.2 Hz, .*HC*.CH_2OH), 5.15–5.05 (3H, *m*, 3 × H–C=C) 4.15 (2H, *d*, *J*=6.9 Hz, CH_2OH), 3.68 (1H, *m*), 2.2–1.9 (10H, *m*), 1.68 (6H, *s*, 2 × Me), 1.60 (9H, *s*, Me), 0.91 (1H, *d*, *J* = 6.6 Hz, OH). The yield in a cold run was 97%.

Isolation of (**1S**)-*cembrene* (**9**) *from a western sulphate turpentine.* Partially purified cembrene-containing turpentine was a gift from Dr Duane F. Zinkel (U.S.D.A. Forest Products Laboratory, Madison, Wisconsin). Vacuum distillation (1–2 mm Hg)

had concd the cembrene peak from 2.5 to 11% and chromatography on silica (elutant, petrol) had increased the cembrene peak to 40%. Purification was continued at Nottingham first by distilling more low bp material at 0.003 mm Hg. (bath 100°) and then by further chromatography of enriched material (~3 g) on silica (Waters Prep. 500, eluting with petrol, bp 40–60°), fractions being monitored by TLC. One fraction was selected and chromatographed further, first on silica, and then thrice more by HPLC (silica, eluting with hexane) to give a reference sample of (1*S*)-cembrene (**9**) of purity >99% by GLC (OV17, temp. programme 130–240°/16° per min) which was used for spectroscopic measurements and comparisons. See Table 3 for ^1H decoupling difference spectrum. ^{13}C NMR δ ($CDCl_3$) 14.3 (2 × Me), 19.9 (2 × Me), 20.8 (Me), 23.5 (CH_2), 26.2 (CH_2), 27.8 (CH_2), 32.8 (CH), 36.5 (CH_2), 38.9 (CH_2), 48.2 (CH), 125.4 (CH), 125.8 (CH), 126.6 (CH), 130.5 (CH), 130.9 (CH), 131.2 (C), 132.5 (C) and 135.2 (C).

all-(E)-[2-^{14}C]-*Geranylgeranoic acid.* The ethyl ester (above) (226.6 mg) was hydrolysed with 2 M-aqueous KOH (2.5 ml) in dioxan (8 ml) at reflux in N_2 overnight. Work-up in the usual way gave *all-(E)*-[2-^{14}C]-geranylgeranoic acid (cf. **6**) (94%), R_f 0.22 (10% ether in hexane) (M^+ 304.2415: Calc. for $C_{20}H_{32}O_2$: M, 304.2401).

(R/S)-[3-^{14}C]-*Cyclic chloroketone* (**16**) $SOCl_2$ (235 μl) was added to a stirred solution of *all-(E)*-[2-^{14}C]-geranylgeranoic acid (195 mg) in dry pyridine (50 μl) and dry C_6H_6 (5 ml) at 0° in N_2, stirring being continued for 2 hr. The organic solution was separated from the white ppt. by needle transfer and the ppt. washed with dry C_6H_6 (2 × 10 ml). Solvents were removed *in vacuo* leaving *all-(E)*-[2-^{14}C]-geranylgeranoyl chloride (99%).

A solution of $SnCl_4$ (25 μl) in dry CH_2Cl_2 (40 ml) was added dropwise to the above acid chloride (206 mg) in anhydrous CH_2Cl_2 (200 ml) at −78° in N_2, and stirring continued for a further hr. The reaction mixture was poured into 7% aqueous $NaHCO_3$ (300 ml) and allowed to attain room temp. The organic phase was then separated, washed with 7% aqueous $NaHCO_3$ (2 × 100 ml), H_2O (2 × 100 ml) and satd brine (100 ml), and then dried (Na_2SO_4) and evapd *in vacuo*. Column chromatography (silica gel; eluting with 10% ether in hexane) gave the (*R/S*)-[3-^{14}C]-cyclic chloroketone (**16**) (100.4 mg, 49%), R_f 0.61 (10% ether in hexane).

In a parallel 'cold' run starting from geranylgeranoic acid (6.01 g), the ketone (**16**) was obtained in 79.5% yield, mp 68.5–70° from ethanol (Lit. [35] mp 69–70°) ν_{max} (nujol) 1678 (C=O) cm^{-1} (M^+ 322.2110. Calc. for $C_{20}H_{31}O^{35}Cl$: M, 322.2063.

(R/S)-[3-^{14}C]-*Cyclic ketone* (**17a**). Tributyltin hydride (100 μl) was added dropwise to a stirred refluxing solution of the [3-^{14}C]-cyclic chloroketone (**16**) (100.4 mg) and azobisisobutyronitrile (3.5 mg) in dry cyclohexane (10 ml) in N_2, and stirring continued (3 hr). After evapn of the solvent the residue was chromatographed on silica gel, eluting with 50% C_6H_6 in hexane, to give the [3-^{14}C]-ketone (**17a**) (79.1 mg, 88%), R_f 0.56 (10% ether in hexane).

In a cold experiment chloroketone (**16**) (2.42 g) was converted into unlabelled (**17a**) (72%), an oil, ν_{max} 1681 (C=O) cm^{-1}, ^{13}C NMR δ ($CDCl_3$): 205.3p.p.m. (C=O), (M^+ 288.2458, Calc. for $C_{20}H_{32}O$: M, 288.2453).

The [3-^{14}C]-*mukulols* (**18a**) *and* (**19a**). Diisobutylaluminium hydride (0.1 M, 5.6 ml) was added dropwise to a stirred solution of the [3-^{14}C]-labelled ketone (**17a**) (79 mg) in dry hexane at 0° in N_2. Stirring was continued at 0° (2 hr) when MeOH (1 ml) was added and stirring continued (15 min) at room temp. The product was filtered and the ppt. washed with warm MeOH (2 × 25 ml). Solvents were evapd *in vacuo* and the product was chromatographed on silica gel (elutant 10% ether in hexane) to give a mixture (54.9 mg, 69%) of the diastereoisomeric [3-^{14}C]-mukulols (**18a**) and (**19a**).

(R/S)-[3-^{14}C]-*Cembrene* (**9**). The mixture of the diastereoisomeric [3-^{14}C]-mukulols (**18a**) and (**19a**) (54.7 mg), dry pyridine (100 μl) and $SOCl_2$ (55 μl) in dry benzene (5 ml) was stirred for 1 hr at 0°. The suspension was diluted with ether (50 ml), washed with aq. $NaHCO_3$ (2 × 25 ml), water (2 × 25 ml) and saturated brine (25 ml). After drying (Na_2SO_4) the solvent was removed *in vacuo* and the residue chromatographed on silica gel eluting with hexane. The mixture of hydrocarbons was then further chromatographed on silica gel containing 20% w/w $AgNO_3$, eluting with 25% benzene in hexane. The (*R/S*)-[3-^{14}C]-cembrene thus isolated was further purified to constant activity by preparative TLC (silica gel, 0.75 mm layer, elutant hexane), (26.6 mg, 52%), R_f 0.69 in hexane. The activity was 28.81 × 10^3 dpm/mg.

tri-n-Butyltin deuteride [36]-Freshly prepared tri-*n*-butyltin hydride (29.1 g) was added portionwise, in N_2, to a stirred solution of cyclohexyl magnesium bromide (0.77 M, 143 ml) in ether containing galvinoxyl (710 mg). After stirring (2 hr) at 20° D_2O (99.8 atom %, 6 ml) was added dropwise at 0°. After further stirring (3 hr) water (100 ml) was then added and the organic layer separated. The aq. phase was extracted with ether and the combined organic solutions dried ($MgSO_4$) and distilled to give tri-*n*-butyltin deuteride (26.4 g, 90%), bp 89–91°/1 mm Hg, n^{20} 1.4722, ν_{max} 1301*s* (Sn-D) cm^{-1} (Found: $(M-{}^2H)^+$ 290.1185 Calc. for $C_{12}H_{27}Sn$ $(M-{}^2H)$ 290.1056).

(R/S)-(3E,7E,11E)-[15-^{2}H]-*Cembra*-3,7,11-*trien*-2-*one* (**17b**). tri-*n*-Butyltin deuteride (2.42 g) was refluxed for 4 hr with the cyclic chloroketone (**16**) (2.47 g) in dry cyclohexane in the presence of azobisisobutyronitrile (33 mg). Evaporation and chromatography gave the (*R/S*)-[15-^{2}H]-ketone (**17b**) (70%), ν_{max} 1682 (C=O) cm^{-1} (Found: M^+ 289.2510. Calc. for $C_{20}H_{31}OD$: M, 289.2516), ^2H NMR δ ($CHCl_3$): 1.74 (*br s*, Me_2C-D). A similar 'cold' experiment is described above.

(±)-(3E,7E,11E)-[15-^{2}H]-*Cembra*-3,7,11-trien-2-ols (**18b**) *and* (**19b**). Diisobutylaluminium hydride (0.15 M in C_6H_6, 0.8 ml) was added to a stirred solution of the (*R/S*)-enone (**17b**) (236 mg, 49%) in benzene (5 ml) at 5°. After 3 hr, methanol (20 ml) was

Table 3. ^1H Decoupling difference spectrum for (1*S*)-cembrene (**9**)

Protons irradiated	Chemical shift	Protons affected
H_3	6.05	
H_5	5.53	6a, 6b, 18-Me
H_2	5.17	3, 1
H_7	5.10	6a, 6b, 19-Me
H_{11}	4.87	10a, 10b, 20-Me
H_{6a}	3.04	5, 7, 6b, 18-Me
H_{6b}	2.40	5, 7, 6a, 19-Me
$H_{10a,b}$	~2.25	
$H_{13a,b}$	~2.00	
$H_{9a,b}$	~2.00	
18-Me	1.79	5, 3, 6a
H_1	~1.6	
19-Me	1.59	6b, 7
20-Me	5.10	11, 13a, 13b
H_{15}	1.48	
$H_{14a,b}$	1.21	13a, b
16-Me	0.85	15
17-Me	0.83	

added. Work-up and chromatography on silica gel eluting with ether–hexane (1:9) separated two diasteroisomers. (±)-Diastereoisomer A (115 mg), an oil, had ν_{max} 3397 cm^{-1} (*br*, OH), (Found: M^+ 291.2698. Calc. for $C_{20}H_{33}OD$), M, 291.2672), ^2H NMR $\delta(CHCl_3)$: 1.78 (*br s* Me_2C-D) ^1H NMR $\delta(CDCl_3)$: 4.60 (1H, *dd*, *J* 9.0, 0.6 Hz, CHOH).

(±)-Diastereoisomer B (15 mg, 6%), an oil had ν_{max} 3400 cm^{-1} (broad OH), (Found: M^+ 291.2664. Calc. for $C_{20}H_{33}OD$, 291.2672), ^2H NMR $\delta(CHCl_3)$ 2.16 (*br s*, Me_2C-D), ^1H NMR $\delta(CDCl_3)$: 4.22 (1H, *dd*, *J* 9.2 Hz, CHOH).

(±)-(3E,7E,11E)-[2-^{2}H]-*Cembra*-3,7,11-*trien*-2-*ols* (**20**) *and* (**21**). $LiAlD_4$ (46 mg) in dry ether (5 ml) was added to the (*R/S*)-trienone (17a) (205 mg) in ether (15 ml) at 0°. After 1 hr, water was added and the organic layer was worked-up and chromatographed on silica gel eluting with ether–hexane (1:9). The major product was the product of 1,4-reduction (47%) as a mixture of diastereoisomers, (Found: M^+ 291.2677. Calc. for $C_{20}H_{33}OD$ M, 291.2672). The mixture showed ν_{max} 1700 (C=O), ^2H NMR $\delta(CHCl_3)$: 1.88 (*br s*, $CDCH_2CO$) and two carbonyls in the ^{13}C NMR spectrum δ214.3 and 216.4.

The second product (22%), ν_{max} 3417–3350 cm^{-1} (*br*, OH), (Found M^+ 291.2666. Calc. for $C_{20}H_{33}OD$, M, 291.2672) had ^2H NMR $\delta(CHCl_3)$: 4.23 (*br s*, CDOH) and thus correspond to (±)-diastereomer B above. The third product (6%), ν_{max} 3380 cm^{-1} (*br*, OH) (Found M^+ 291.2672. Calc. for $C_{20}H_{33}OD$, M, 291.2672) had ^2H NMR $\delta(CHCl_3)$: 4.60 (*br s* CDOH) and thus corresponds to (±)-diastereomer A above.

R/S-[2^2-H]-*Cembrene* (cf. **9**). [2-^{2}H]-Diastereoisomer B above (53 mg) in dry pyridine (92 mg) and C_6H_6 (4 ml) was treated with $SOCl_2$ (74 mg) at 5° for 2 hr. Work-up and chromatography on silica gel, eluting with *n*-hexane gave a mixture of hydrocarbons (26 mg). Further separation using TLC on silica gel impregnated with 20% w/w $AgNO_3$, eluting with *n*-hexane–C_6H_6 (4:1), gave (*R/S*)-[2-^{2}H]-cembrene (cf. **9**) (21 mg). (Found: M^+ 273.2559. Calc. for $C_{20}H_{31}D$, M 273.2567). ^2H NMR $\delta(CHCl_3)$: 5.23 (*br s* C=C-D), ^{13}C NMR $\delta(CDCl_3)$: 14.3 (Me), 14.4 (Me), 19.85 (Me), 19.9 (Me), 20.8 (Me), 23.6 (CH_2), 26.3 (CH_2), 27.8 (CH_2), 32.9 (CH), 36.6 (CH_2), 38.95 (CH_2), 48.2 (CH), 125.4 (CH), 125.8 (CH), 126.65 (CH), 130.45 (CH), 130.9 (CD), 131.3 (C), 132.6 (C), and 135.2 (C).

(R/S)-[15-^{2}H]-*Cembrene* (cf. **9**). [15-^{2}H]-Diastereoisomer A of the cembratrienol above (106 mg) in dry pyridine (186 mg) and C_6H_6 was dehydrated using $SOCl_2$ (149 mg) at 5° for 2 hr. Work-up and chromatography gave a mixture of hydrocarbons (57 mg) further purified by argentation chromatography as for the [2-^{2}H]-case above to give (*R/S*)-[15-^{2}H]-cembrene (cf. **9**) (45 mg) (Found: M^+ 273.2553. Calc. for $C_{20}H_{31}D$, M, 273.2567), ^2H NMR $\delta(CDCl_3)$: 1.50 (*br s*, Me_2C-D). The ^{13}C NMR spectrum ($CDCl_3$) was closely similar to that for cembrene except for a triplet signal in the proton decoupled spectrum at δ32.35 assigned to 15-C-D.

all-trans-*Geranylgeranoic acid* (*cf.* **6**) *from natural* all-trans *geranylgeraniol* (**3**). Crude alcohol (**3**) from extraction of *Bixa orellana* seed was purified *via* acetylation, chromatography and hydrolysis (aq. K_2CO_3). Pure geranylgeraniol (2.3 g) was oxidised to geranylgeranial (2.31 g, almost quantitative) by stirring with active MnO_2 (14.2 g) in hexane at 0°. The aldehyde (Found: M^+ 288.2441. Calc. for $C_{20}H_{32}O$, M, 288.2453) had the expected ^1H and ^{13}C NMR data, showing an aldehyde proton at $\delta(CDCl_3)$: 10.05 (1H, *d*, *J* = 7.8 Hz). Dry NaCN (1.96 g) was added to the aldehyde (1.16 g) in dry methanol at 0°. After 30 min freshly prepared neutral silver (II) oxide (9.92 g) was added portionwise and the mixture was stirred at 0° (2 hr). Work-up by concn and extraction with ether, washing the ethereal solution with 10% aq. formaldehyde, then H_2O and brine, gave on drying and evapn an oil. The latter was chromatographed on silica gel, eluting with ether–hexane (1:9) to give *all-trans*-geranylgeranoic acid (cf. **6**) (1.1 g, 91%), spectroscopically identical with the specimen described above.

Reduction of poilaneic acid methyl ester (**23a**). Poilaneic acid [33] (**22**), mp 94–95°, $[\alpha]^{25}$-136.8° ($CHCl_3$) (208 mg) in $CHCl_3$ (3 ml) was esterified by the addition of CH_2N_2 in ether at 0°C. Evapn and chromatography on silica gel [elutant ether–hexane (9:1)] gave methyl poilaneate (**23a**) (195 mg, 90%), (Found: M^+ 316. Calc. for $C_{21}H_{32}O_2$ M, 316). ^1H NMR ($CDCl_3$) δ: 6.07 (1H, *d*, *J* = 15.8 Hz, 3-H) 5.85 (1H, *t*, *J* = 5.4 Hz, 11-H), 5.57 (1H, *t*, *J* = 7.5 Hz 5-H), 5.20 (1H, *dd*, *J* = 15.8, 10.8 Hz, 2-H), 5.16 (1H, *d*, *J* = 10.8 Hz, 7-H), 3.74 (3H, *s*, CO_2Me), 1.81 (3H, *s*, 10-Me), 1.67 (3H, *s*, 19-Me), 3.2–1.3 (12H, *m*), 0.83 (3H, *d*, *J* = 7.3 Hz, Me-CHMe), 0.80 (3H, *d*, *J* = 7.3 Hz, MeCH-Me). The ester (195 mg) in dry ether (20 ml) was treated with $LiAlH_4$ (98 mg). After stirring (1 hr) the reaction was quenched and filtered and the filtrate was evapd to give the alcohol (**23b**) (171 mg, 91%), mp 90–91° (Lit. [33] mp 92–94°). (Found: M^+ 288.2457. $C_{20}H_{32}O$ requires 288.2453). ^1H NMR ($CDCl_3$) δ: 6.04 (1H, *d*, *J* = 15.5 Hz, 3-H), 5.55 (1H, *dd*, *J* = 7.1, 7.4 Hz, 5-H), 5.19 (1H, *q*, *J* = 15.6, 9.2 Hz, 2-H), 5.15–5.08 (2H, *m*, 7-H and 11-H), 4.27 (1H, *d*, *J* = 12.1 Hz, 20-H), 4.00 (1H, *d*, *J* = 12.1 Hz, 20-H'), 3.04 (1H, *m*, 1-H), 2.5–1.2 (11H, *m*), 1.8 (3H, *s*, 18-Me), 1.59 (3H, *s*, 19-Me), 0.87 (3H, *d*, *J* = 7.5 Hz), Me.CHMe), 0.83 (3H, *d*, *J* = 7.5 Hz, Me.CH.Me).

The ^{13}C NMR spectrum for poilaneic acid was partially assigned as follows, $\delta(CDCl_3)$: 47.9 (C-1), 131.3 (C-2), 130.4 (C-3), 130.9 (C-4), 125.6 (C-5), 128.0 (C-7), 128.7 (C-8), 147.5 (C-11), 135.1 (C-12), 29.5 (C-14), 32.8 (C-15), 21.0 (C-16), 19.3 (C-17), 20.0 (C-18), 14.5 (C-19), 173.5 (C-20) ppm. Signals at 38.5, 32.1, 26.2 and 25.9 correspond to C-6, 9, 10 and 13 but require further evidence for assignment. See Table 4 for ^1H decoupling difference spectrum for the acid.

The C_{24}*-hydrocarbon* (**26**). 20-Hydroxy *ent*-cembrene (**23b**) (15 mg) was treated with PBr_3 (1.6 μl) in ether (2 ml) at 0° for 30 min and at 20°C for 30 min. TLC indicated that all the starting alcohol had been converted and the bromide (**23f**) was isolated by quenching with $NaHCO_3$ and extraction with ether and evapn. The crude bromide in THF (2 ml) at −78° was treated with *n*-butyl lithium (1.47 M, 0.2 ml) and stirred at −78°

Table 4. ^1H Decoupling difference spectrum for poilaneic acid (**22**)

Protons irradiated	Chemical shift	Protons affected
H_3, H_{11}	~6.05	**2**, 10a, 10b
H_5	5.58	6a, 6b, 18-Me
H_2	5.21	3, 1
H_7	5.17	6a, 6b, 19-Me
H_{6a}	3.07	5, 7, 6b, 18-Me > 19-Me
H_{10a}	2.92	11, 9a, 9b, 10b
H_{14a}	2.55	13a, 14b
H_{9a}, H_{6b}	2.47	**11**, **10a**, **9b**, **10b**, **19**-Me; 5, 6a
H_{9b}	2.22	10a, 9a, 10b
H_{10b}, H_{13a}	2.01	**11**, **10a**, **9a**; 14a, 13b, 14b
H_{13b}	~1.8	14a, 13a, 14b
18-Me	1.81	5, 6a
H_1	1.74	3, 2, 14a, 15, 14b
19-Me	1.65	7, 6a, 9a or 6b, 9b
H_{15}	1.49	1, 16-Me, 17-Me
H_{14b}	1.35	14a, 13a, 13b, 15
16, 17-Me	0.84	15

for 1 hr. D_2O (0.5 ml) was added and the mixture was allowed to warm to room temp. and extracted. Purification by TLC on silica [eluant petrol (bp 40–60°)] (R_f 0.73) gave the C_{24}-hydrocarbon (**26**) (52%). (Found: M^+ 328.3125. $C_{24}H_{40}$ requires M, 328.3130). There were losses of −43 (C_3H_7) (*m/z* 285.2551. $C_{21}H_{33}$ requires *m/z* 285.2582) and −57 (C_4H_9) (*m/z* 271.2420. $C_{20}H_{31}$ requires *m/z* 271.2425).

Aldehyde (**23d**). CrO_3 (200 mg, 0.15 mmol) was added to pyridine (0.5 ml) in CH_2Cl_2 (20 ml) at 0°, stirred (30 min) and cooled to 0°. Alcohol (**23b**) (94 mg, 0.33 mmol) in CH_2Cl_2 (1 ml) was added and stirred at 0° (1 hr) and 20° (1 hr). Work-up followed by chromatography on silica gel eluting with ether–hexane (3:17) gave the aldehyde (**23d**) (93 mg, almost quantitative) ^{1}H NMR ($CDCl_3$) δ: 10.09 (1H, CHO), 6.4–6.3 (1H, *m*, 11-H), 6.01 (1HH, *d*, J = 15.5 Hz, 3-H), 5.57 (1H, *t*, J = 7.8 Hz, 7-H), 5.20 (1H, *q*, J = 15.6, 9.6 Hz, 2-H), 5.25–5.1 (1H, *m*, 5H), 3.15–2.9 (1H, *m*, 1-H), 2.8–1.2 (11H, *m*), 1.81 (3H, *d*, J = 1.3 Hz, 18-Me), 1.65 (3H, *s*, 19-Me), 0.80 (3H, *d*, J = 6.4 Hz, MeCH.Me), 0.78 (3H, *d*, J = 6.5 Hz, Me.CHMe. On a silica gel plate [elution 30% ether in petrol (bp 40–60°] the aldehyde had R_f 0.52 (alcohol R_f 0.25). This oxidation was also carried out with $Bu_4N^+CrO_3Cl^-$ (75% yield) and pyridinium chlorochromate (90% yield).

Reduction of aldehyde (**23d**). Cerium trichloride nonahydrate (10 mg, 0.027 mmol) was stirred with aldehyde (**23d**) (7.7 mg, 0.027 mmol) in MeOH (3 ml) for 5 min. $NaBH_4$ (2.1 mg, 0.054 mmol) was added, stirred (10 min), and H_2O was then added. Extraction and plate chromatography [eluting with ether–hexane (1:5)] gave alcohol (**23b**) (5.6 mg) (Found: M^+ 283.2457. $C_{20}H_{32}O$ requires M 288.2453). The NMR spectrum was identical with that above.

A similar reduction of the aldehyde (90 mg) using sodium borotritide (initial activity 20 mCi), stirring at 0° for 30 min followed by $NaBH_4$ (12 mg) gave, on work-up and chromatography on Woelm silica [elution 20% ether in light petroleum (bp 40–60°), (1*R*)-[20-^{3}H]-20 hydroxycembrene (**25**) (60 mg) as a white solid having a specific activity of 0.12 mCi/mg. No 1,4-reduction was observed in this experiment.

Treatment of alcohol (**23b**) *with methanesulphonyl chloride.* Methanesulphonyl chloride (15 μl) was added to a stirred mixture of pyridine (8 μl) and alcohol (**23b**) (15 mg) in CH_2Cl_2 (1 ml). After stirring (24 hr), the product was isolated (8 mg) and purified by TLC (elutant hexane), to give the chloride (**23e**) (Found: M^+ 306, $C_{20}H_{31}Cl$ requires M 306). ^{1}H NMR ($CDCl_3$) δ: 5.99 (1H, *d*, J = 15.5 Hz, 3-H), 5.55 (1H, *t*, J=7.3 Hz, 5-H), 5.19 (1H, *q*, J=15.2, 9.6 Hz, 2-H), 5.25–5.05 (2H, *m*, 7-H and 11H), 4.14 (1H, *d*, J=10.8 Hz, CHHCl), 3.97 (1H, *d*, J = 10.9 Hz, CHHCl), 3.06 (1H, *m*, 1-H), 2.5–1.1 (11H, *m*), 1.80 (3H, *t*, J = 1.3 Hz, 18-Me), 1.61 (3H, *s*, 19-Me), 0.85 (3H, *d*, J=9.8 Hz, MeCH.Me), 0.82 (3H, *d*, J=9.8 Hz, MeCH.Me).

Reduction of the chloride (**23e**). $LiAlH_4$ (20 mg) was added to the chloride (6 mg) in ether (2 ml) at 0° and stirred for 3 hr at 20°. Wet ether was added and the product was worked-up and purified by silica plate chromatography (elutant hexane) to give (1*R*)-cembrene (24) (4 mg, 75%), (Found: M^+ 272.2535 $C_{20}H_{32}$ requires 272.2504). ^{1}H NMR ($CDCl_3$) δ: 6.05 (1H, *d*, J = 15.5 Hz, 3-H), 5.53 (1H, *dd*, J = 7.2 Hz, 5-H), 5.17 (1H, *q*, J = 15.5, 9.6 Hz, 2-H), 5.15–5.05 (1H, *m*, 7-H), 4.87 (1H, *d*, J = 6.2, 11-H), 3.05 (1H, *m*, 1-H), 2.5–1.2 (11H, *m*), 1.79 (3H, *t*, J = 1.3 Hz, 18-Me), 1.59 (3H, *s*, 19-Me), 1.51 (3H, *s*, 20-Me), 0.86 (3H, *d*, J = 6.8 Hz, Me.CHMe), 0.83 (3H, *d*, J = 6.8 Hz, MeCH.Me).

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