201. A Chemical Study of *Virginia* Tobacco Flavour (*Nicotiana Tabacum* L.)
I. Isolation and Synthesis of Two Bicyclodamascenones

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Summary. Aged *Virginia* tobacco was steam-distilled and the resulting condensate investigated by chromatographic methods. This allowed identification of the bicyclodamascenones **A** and **B** in a small subfraction from this condensate. Both these ketones, which are novel tobacco constituents, were also synthesized and a possible mechanism is proposed for their formation *via* acid-catalysed cyclization of β-damascenone (**1**).

Virginia and *Burley*[1]) tobaccos are among the most important raw materials used for the manufacture of cigarette products. They exhibit properties directly related to the curing process they have undergone. *Burley* tobacco is air cured, dark coloured, and practically devoid of soluble carbohydrate, while *Virginia* tobacco is flue cured, bright, and contains much invert sugar [2]. Each type of curing induces many specific, fundamental biochemical and chemical changes in the tobacco leaf, thus leading to a typical aroma after fermentation and ageing. Since *Virginia* tobacco is known to be more acidic than *Burley*[1]) tobacco [3], one may expect its flavour to contain a number of specific constituents arising from acid-catalysed cyclizations, aldol condensations, etc. The present study of *Virginia* tobacco flavour will, we hope, allow this assumption to be tested, and enable the results of other similar investigations [4][2]) to be extended.

1. *Preparation of* Virginia *tobacco condensate*[3]). Steam-distillation of a high-grade aged, chopped *Virginia* tobacco (99.2 kg) yielded 1800 l of an aqueous distillate which was acidified to pH 3–4 with 50% sulfuric acid and extracted four times with chloroform. This afforded 174 g of a tobacco oil practically free of nicotine, but presenting excellent organoleptic properties. The residual aqueous layer, brought to pH 8.5 with sodium hydrogencarbonate and extracted with chloroform (3×), gave a second, basic extract weighing 46 g. Thus, the total yield of *Virginia* tobacco condensate was 0.22% by weight of starting tobacco.

2. *Identification of the bicyclodamascenones* **A** *and* **B**. *Virginia* tobacco condensate was separated in a classical way according to the *Scheme*. Among the various fractions obtained, VN2 exhibited typical organoleptic properties[4]) prompting further investigation by silica gel chromatography. The resulting subfractions VN2(d) and VN2(e)

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1) For our previous study of *Burley* tobacco flavour see [1] and preceding papers.

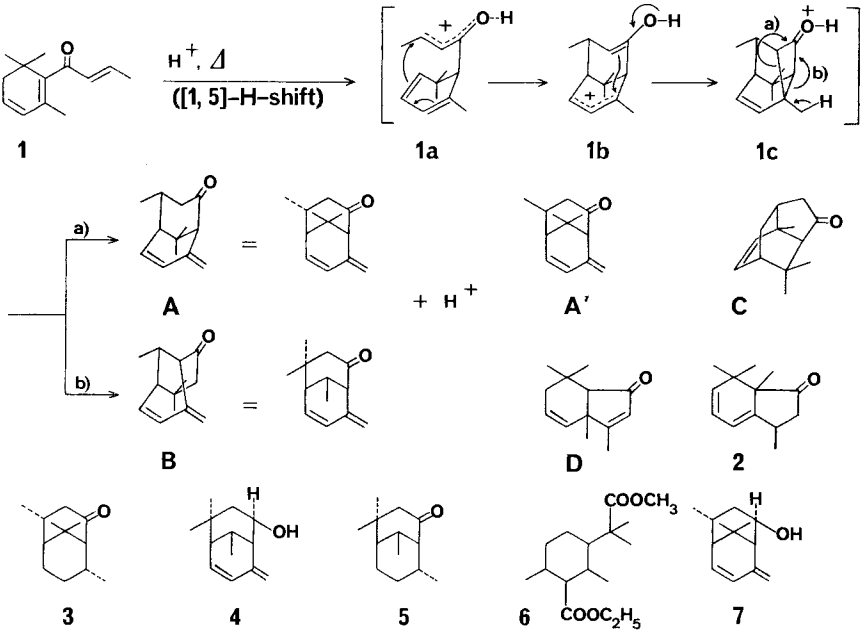
2) We thank Dr. *D. L. Roberts et al.* (*R. J. Reynolds Tobacco Company*) for having informed us of their results prior to publication.

3) The preparation of *Virginia* tobacco condensate was carried out at our pilot laboratory under the supervision of Dr. *H. Strickler*, to whom we express our thanks.

4) We are indebted to Dr. *P. Dietrich* and Mr. *A. Y. Smith* (*Firmenich SA*, Geneva) for having kindly evaluated the flavouring properties of these fractions.

were in turn selected for final separation by GLC. (gas liquid chromatography) using semi-preparative 'polar' (Carbowax) and 'non-polar' (silicone oil) columns, as well as a capillary column (Ucon 5100, 50 m × 0.3 mm) for monitoring the semi-preparative separations. Each compound collected was examined by the usual spectral methods. This permitted the identification (MS., ^{1}H-NMR.) of the *bicyclodamascenones* **A** [5] and **B** in subfractions VN2(d) and VN2(e) (total content 0.1%). They were accompanied by the constituents β-damascenone (**1**) [6], solanone [7], solanofuran [8], β-cyclocitral [9], etc.[5]).

The bicyclodamascenones **A** and **B** were possibly formed in *Virginia* tobacco at the curing and/or ageing stages, either by acid-catalysed cyclization of β-damascenone (**1**) (favoured by the relative acidity of this type of tobacco), or by an alternative enzymatic process. To settle the question, it would be desirable to check the optical activity of these ketones, but this has so far been impossible due to material shortage.

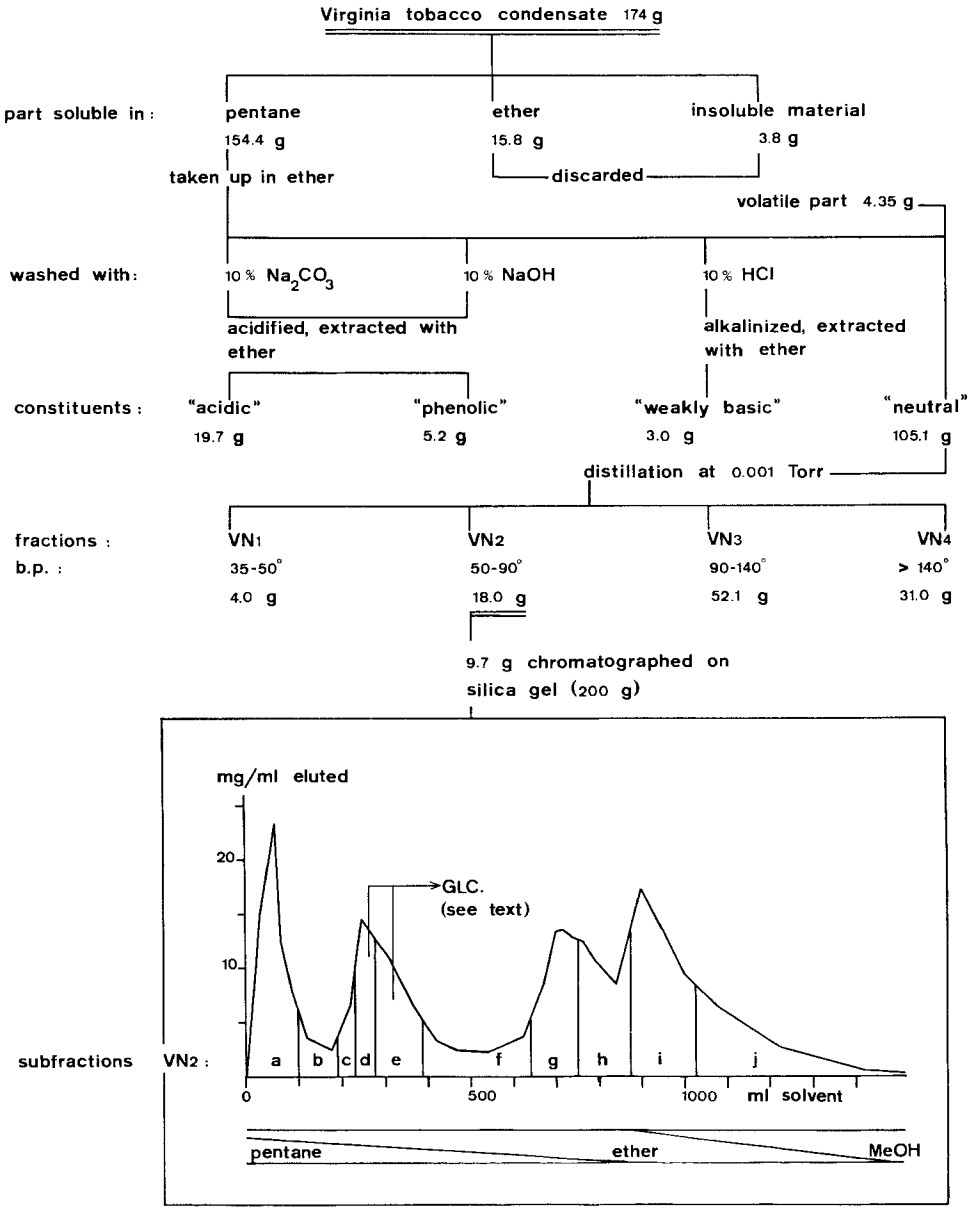


3. *Synthesis and structural study of the bicyclodamascenones* **A** *and* **B**. The cyclization of β-damascenone (**1**) proceeded easily at 180° under N_2 in the presence of 1% *p*-toluenesulfonic acid [5]. There was thus obtained in a yield of 82% a mixture of ketones **A** [5] (44%), **C** [10] (14%), and **D** [5] (7%), accompanied by the *novel isomer* **B** (35%). The major bicyclodamascenones **A** and **B**, separated from the mixture by selective semicarbazone formation and purified by semi-preparative GLC., had the following characteristics.

A. IR. (neat, bands with decreasing intensities): 1700, 1220, 880, 1130, 1625, 1585, 3100 cm^{-1}. – UV. (EtOH): λ_{max} = 232 nm (ε = 14700). – MS. (*m/e* (% relative abundance)): 69 (99), 91 (46), 105 (100), 119 (92), 120 (48), 146 (65), 148 (70), 190

[5]) Full details will be published later.

Scheme. *Investigation of nicotine-free* Virginia *tobacco condensate*



(M^+, 48). – ^{1}H-NMR. (90 MHz, $CDCl_3$, δ-values): 0.98 (*s*, 6H); 1.00 (*d*, $J = 6$, 3H); 1.9–2.2 (*m*, 3H); 2.2–2.7 (*m*, 1H); 2.80 (br. *s*, 1H); 5.02 (*s*, 2H); 5.94 (*d*×*d*, $J = 9$, $J' = 6.5$, 1H); 6.35 (*d*, $J = 9$, 1H).

B. IR. (neat): 1705, 1220, 885, 1625, 1590, 3100 cm^{-1}. – UV. (EtOH): $\lambda_{max} =$ 234 nm ($\varepsilon = 16230$). – MS.: 77 (10), 83 (19), 91 (96), 105 (26), 106 (100), 175 (10), 190 (M^+, 15). – ^{1}H-NMR.: 0.92 (*d*, $J = 7$, 3H); 1.04 (*s*, 6H); 2.12 (*q*-like *AB*-system,

$J = 14$, 2H); 1.7–2.6 (*m*, 2H); 2.98 (br. *s*, 1H); 5.04 (*d*, $J \simeq 2$, 2H); 5.97 ($d \times d$, $J = 10$, $J' = 5.5$, 1H); 6.33 (*d*, $J = 10$, 1H).

Contrary to our preliminary report [5], no methyl-rearranged cyclopentanone **2** was formed during the above acid-catalysed cyclization of β-damascenone (**1**). Our former cyclopentanone must in fact have been impure **C**, as demonstrated by the present study in which more powerful separation techniques were employed.

The two major bicyclodamascenones described above were expected to have the epimeric structures **A** and **A'** resulting from a non-stereoselective cyclization of the intermediate hydroxyallyl cation **1a**. That bicyclodamascenone **A** was one of these epimers could be deduced from its spectral data (*vide supra*) and from the ^{1}H-NMR./Eu(fod)$_3$ spectrum of the related tetrahydro-ketone **3**. The latter spectrum indeed exhibited a typical *ABX* coupling pattern consistent with the grouping $>$CH–CH$_2$–CO– ($J_{AB} = 18$, $J_{AX} = 11$, $J_{BX} = 7$) (see exper. part). Most unexpectedly, however, no such *ABX* coupling appeared in the ^{1}H-NMR. spectrum of the second bicyclodamascenone isomer. Instead, there was only a *q*-like *AB*-system ($J = 14$) at 2.12 ppm which ruled out either structures **A** or **A'** and suggested the rearranged structure **B** for this ketone (see the spectral data above). This rearranged structure was further supported by the ^{1}H-NMR. spectrum of the related axial alcohol **4**, in which the methylene protons of $\geqslant$C–CH$_2$–CHOH– gave rise to a ($d \times q$)-like *AB*-system ($J = 15$, $J' = 4$) at 1.34 ppm, collapsing to a *q*-like *AB*-system ($J = 15$) upon irradiation of the –C*H*OH– proton (at 3.86). Still another indication of the rearranged structure **B** was gained by oxidizing the related tetrahydro-ketone **5** with peroxyacid under energetic conditions [11]. The structure of the resulting diester **6** was confirmed by ^{1}H-NMR. (see exper. part).

4. *Mechanism of the acid-catalysed cyclization of β-damascenone* (**1**) *to* **A** *and* **B**. The formation of the bicyclodamascenones **A** and **B** from β-damascenone (**1**) can be rationalized *via* stereospecific cyclization of the '*trans*' hydroxyallyl cation **1a** leading successively to **1b** and to the protonated cyclobutanone **1c**. Proton elimination and cleavage of either the a) or b) bond of the cyclobutanone ring in **1c** would then furnish **A** and **B**. The respective *endo* and *anti* configurations of the resulting bicyclodamascenones were corroborated by the ^{1}H-NMR. spectra of the related axial alcohols **7** and **4**. In both cases, only one methyl group (at a tertiary C-atom) exhibited substantial deshielding proving its *cis*-relationship to the OH function (1,3-diaxial interaction) (see exper. part).

Experimental part

The spectra were obtained with the instruments already described [8] (the mass spectra were determined at 70 eV, inlet temperature 150°; the ^{1}H-NMR. spectra were measured in $CDCl_3$). The GLC. separations were performed on the gas chromatographs mentioned in [1]. All liquid-solid chromatographic separations were carried out on 0.05–0.2 mm silica gel for column chromatography (*Merck AG.*).

1. *Bicyclodamascenones* **A** *and* **B**. A stirred mixture of β-damascenone (**1**) [6] (39.8 g, 209 mmol) and *p*-toluenesulfonic acid (0.398 g) was heated for 75 min at 180° under nitrogen. The product cooled to 20° was taken up in ether and washed successively with 5% sodium hydrogen carbonate (1×) and brine (3×). Distillation at 0.001 Torr afforded 32.93 g (82%) of a mixture (b.p. 55–82°) of ketones **A** (44%), **B** (35%), **C** (14%), and **D** (7%). Relative retention times (Ucon 5100, 120°, 50 m × 0.3 mm capillary column): 1.00 (**C**), 1.02 (**B**), 1.09 (**A**), 1.24 (**D**). The mixture of ketones **A**–**D** (40.95 g, 215 mmol) was refluxed for 64 h in ethanol (250 ml) and water (200 ml) in the pres-

ence of semicarbazide hydrochloride (23.93 g, 214 mmol) and sodium acetate trihydrate (35.87 g, 264 mmol). After ethanol removal *in vacuo*, the mixture was extracted 2× with ether and washed with 10% hydrochloric acid (1×), 5% sodium hydrogencarbonate (2×), brine (3×). After the usual work-up the resulting crude semicarbazone mixture was steam-distilled, yielding 1.25 l of aqueous condensate whose extraction with ether afforded 15.26 g of a mixture of **A** (26%), **B** (36%), **C** (26%), and **D** (12%) (GLC.). The steam-distillation was then resumed in the presence of oxalic acid (100 g) until a second portion of 2.25 l of aqueous condensate was collected. The crude product (18.88 g) obtained by extracting the latter with ether was distilled (0.001 Torr): Fr. 1, b.p. 61–68°, 0.666 g; Fr. 2, b.p. 68–73°, 16.633 g. GLC. indicated Fr. 2 to be a 56:44 mixture of bicyclodamascenones **A** and **B** which were finally separated by semi-preparative GLC. (15% Carbowax). For the spectral data see theor. part, *Section 3*.

2. *Tetrahydro-bicyclodamascenones* **3** *and* **5**. The catalytic hydrogenation of an ~1:1 mixture of **A** and **B** (900 mg) proceeded very slowly in the presence of Pt (90 mg PtO_2) and acetic acid (9 ml) at 20°/730 Torr. Several further portions of PtO_2 had to be added (total 1 g). After 72 h of hydrogenation, the usual work-up afforded 830 mg of a mixture of five compounds (GLC.: Ucon 5100, 120°, 50 m × 0.3 mm capillary column; relative retention times: 1.00 (45%), 1.32 (6%), 1.57 (40%), 1.68 (4%), 2.00 (5%)). Chromatography of this mixture on silica gel (30 g) in the presence of toluene containing increasing amounts of ethyl acetate permitted the elution of pure ketone **3** (150 mg), followed by an intermediate fraction (580 mg), and by a slightly impure alcohol (80 mg) related to ketone **5**. This alcohol was stirred for 2 h at 20° in ether (0.5 ml) with *Jones* reagent (0.3 ml) [12]. The usual work-up afforded 62 mg of ketone **5** containing about 5% of isomer **3**. Final purification of **5** could be achieved by semi-preparative GLC. (5% Carbowax).

Tetrahydro-bicyclodamascenone **3**. – IR. (neat): 1695, 1450, 1220, 1380, 1110. – MS.: 41 (39), 55 (31), 69 (84), 81 (29), 83 (38), 109 (30), 121 (21), 179 (100), 194 (M^+, 20). – ^{1}H-NMR.: 0.77 (*d*, J = 7, 3H); 0.90 (*s*, 3H); 1.02 (*d*, J = 6.5, 3H); 1.10 (*s*, 3H); 1.1–2.7 (*m*, 10H). The methylene protons $-CH_2-CO-$ appeared as the *AB* part of an *ABX* system when shifted downfield by addition of Eu(fod)$_3$. The actual parameters observed were δH_A = 4.75 (J_{AB} = 18, J_{AX} = 11); δH_B = 5.25 (J_{BA} = 18, J_{BX} = 7); the latter signal overlapped with a *d* (J = 5) at 5.36 due to the bridgehead *methine* proton located α to the carbonyl function.

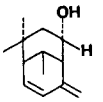
Tetrahydro-bicyclodamascenone **5**. – IR. (neat): 1695, 1230, 1460, 1380, 1480. – MS.: 41 (41), 55 (31), 69 (32), 83 (98), 84 (37), 95 (32), 109 (57), 179 (100), 194 (M^+, 13). – ^{1}H-NMR.: 0.78 (*d*, J = 6.5, 3H); 1.02 (*s*, 3H); 1.08 (*s*, 3H); 1.12 (*d*, J = 6, 3H); 1.1–2.6 (*m*, 10H).

3. *Diester* **6**. The intermediate fraction (580 mg) from the preceding chromatography of the hydrogenated derivatives of **A** and **B** was taken up in ether (2 ml) and stirred for 3 h at 20° with *Jones'* reagent (1 ml) [12]. Usual work-up afforded a mixture (470 mg) of ketones **3** (50%) and **5** (40%), accompanied by two minor impurities (10%) (GLC.). This mixture (450 mg, 2.32 mmol) was reluxed for 20 h in chloroform (6 ml, *stabilized with ethanol*) in the presence of *m*-chloroperbenzoic acid (570 mg, 2.81 mmol, 85% pure) and *p*-toluenesulfonic acid (10 mg). After solvent removal *in vacuo*, the product was taken up in ether and washed with 10% sodium carbonate (2×), 5% sodium hydroxide (2×), and water. This afforded 210 mg (> 90%) of recovered ketone **3** (previous experiments had demonstrated the inertness of this ketone towards *m*-chloroperbenzoic acid). The alkaline washings were acidified and extracted with ether, giving a crude acidic fraction which was esterified with diazomethane. Partial distillation of the resulting ester mixture under reduced pressure allowed removal of most of the methyl *m*-chlorobenzoate present. The remaining crude diester **6** (150 mg) was then purified by semi-preparative GLC. – IR. (neat): 1725, 1140, 1160, 1205, 1260. – MS.: 41 (22), 102 (47), 109 (100), 183 (17), no discernible M^+. – ^{1}H-NMR.: 0.70 (*d*, J = 5, 3H); 0.87 (*d*, J = 7.5, 3H); 1.09 (*s*, 3H); 1.11 (*s*, 3H); 1.22 (*t*, J = 7, 3H); 1.3–2.0 (*m*, 6H); 2.0–2.4 (*m*, 2H); 3.63 (*s*, 3H); 4.10 (*q*, J = 7, 2H). The relative deshielding exhibited by the two methyl groups at a tertiary C-atom is consistent with structure **6**.

The formation of this mixed diester suggests that a reactive species such as an anhydride appeared during the above oxidation of ketone **5** and trapped the ethanol present in the chloroform used. The intermediate acid-ester thus formed yielded diester **6** upon subsequent treatment with diazomethane.

4. *Bicyclodamascenols* **4** *and* **7**. A slurry of lithium aluminium hydride (0.250 g, 6.5 mmol) in anhydrous ether (25 ml) was refluxed for 45 min and 15 ml of the resulting solution were intro-

duced over 15 min at 20° into a 56:44 mixture of bicyclodamascenones **A** and **B** (4.75 g, 25 mmol) in anhydrous ether (125 ml)[6]. The mixture was refluxed for $2^1/_2$ h, cooled, decomposed by addition of several drops of water, filtered, and the filtrate concentrated to dryness. There were obtained ~4.5 g of a mixture containing ketones **A** (52%) and **B** (8%), the axial bicyclodamascenols **4** (17%) and **7** (9%), and the equatorial epimer **8** (14%) (GLC.: Ucon 5100, 120°, 50 m × 0.3 mm capillary column; relative retention times: 1.00 (**B**), 1.07 (**A**), 1.19 (**8**), 1.85 (**4**), 1.98 (**7**)).



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The reduction mixture was chromatographed on silica gel (235 g) in the presence of ether/hexane 1:5, whereby the unreacted ketones **A** and **B** were eluted first. The next fractions consisted of bicyclodamascenol **4** (173 mg), a ~1:1 mixture of **4** and **7** (926 mg), and the equatorial epimer **8** (165 mg). After further purification by semi-preparative GLC., the bicyclodamascenols **4**, **7**, and **8** exhibited the spectral characteristics described below. In each case, the structures of these alcohols were confirmed by oxidation with *Brown*'s reagent [13] to the parent ketones **A** or **B**.

Bicyclodamascenol **4**. – IR. (neat): 880, 3430, 1025, 3050, 1000, 800, 1590, 1630, 3100. – MS.: 41 (35), 85 (57), 91 (86), 93 (42), 105 (42), 106 (92), 107 (100), 118 (51), 121 (32), 192 (M^+, 28). – ^{1}H-NMR.: 0.83 (*s*, 3H); 0.86 (*d*, J = 7, 3H); 1.24 (*s*, 3H); 1.34 ((*d* × *q*)-like *AB*-system, J = 15, J' = 4, 2H); 1.40 (*s*, 1H, OH); 1.77 (*d*, J = 6, 1H); 2.32 (br. *s*, 1H); 2.52 (*m*, 1H); 3.86 (br. *s*, 1H); 4.86 (*s*, 1H); 4.95 (*s*, 1H); 5.72 (*d* × *d*, J = 10, J' = 6, 1H); 6.17 (*d*, J = 10, 1H).

Bicyclodamascenol **7**. – IR. (neat): 3340, 880, 1055, 1625, 1590, 3100. – MS.: 41 (40), 71 (51), 91 (50), 105 (81), 107 (89), 121 (100), 133 (52), 148 (67), 159 (40), 177 (17), 192 (M^+, 44). – ^{1}H-NMR.: 0.88 (*s*, 3H); 0.85 (*d*, J = 7, 3H); 1.30 (*s*, 3H); 1.3–1.5 (*m*, 3H); 1.85 (*m*, 1H); 2.0–2.5 (br. *m*, 1H); 2.09 (br. *s*, 1H); 3.86 (narrow *m*, 1H); 4.84 (br. *s*, 1H); 4.90 (br. *s*, 1H); 5.68 (*d* × *d*, J = 10, J' = 6, 1H); 6.21 (*d*, J = 10, 1H).

Bicyclodamascenol **8**. – IR. (neat): 880, 1030, 1050, 1075, 1450, 3450, 3050, 1630, 1595, 3100. – MS.: 41 (34), 85 (58), 91 (77), 93 (49), 106 (59), 107 (100), 118 (39), 192 (M^+, 35). – ^{1}H-NMR.: 0.90 (*d*, J = 7, 3H); 0.90 (*s*, 3H); 1.10 (*s*, 3H); 1.25–1.80 (*m*, 4H); 2.07 (*m*, 1H); 2.42 (narrow *m*, 1H); 3.83 (br. *m*, 1H); 4.88 (*s*, 1H); 5.10 (*d*, $J \simeq 1.5$, 1H); 5.77 (*d* × *d*, J = 10, J' = 6, 1H); 6.28 (*d*, J = 10, 1H).

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[6]) This reduction was effected with only 62% of the theoretically required amount of lithium aluminium hydride in order to demonstrate the relatively higher reactivity of bicyclodamascenone **B**.