Pergamon

Phytochemistry, Vol. 45, No. 6, pp. 1207 1212, 1997

0031-9422/97 $17.00+0.00

PII: S0031-9422(97)00124-6

PRENYLATED PHENYLPROPENES FROM *COLEONEMA PULCHELLUM* WITH ANTIMICROBIAL ACTIVITY

GÜNTER BRADER, MARKUS BACHER,* OTMAR HOFER* and HARALD GREGER†

Comparative Phytochemistry Department, Institute of Botany, University of Vienna, Rennweg 14, A-1030 Wien, Austria; *Institute of Organic Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Wien, Austria

(*Received* 14 *October* 1996)

Key Word Index—*Coleonema pulchellum*; Rutaceae; roots, prenylated phenylpropenes; tetrahydrofurofuran lignans; diterpenes; alkamides; antimicrobial activity.

Abstract—The lipophilic root extract of *Coleonema pulchellum* was analysed and tested for antifungal and antibacterial activity. Eight previously undescribed prenyloxy and geranyloxy phenylpropenes, were isolated as major compounds together with the known evofolin-C as well as the lignans (±)-sesamin and (±)-prenylpiperitol, the diterpene (−)-pimara-9(11),15-dien-19-oic acid and the 2,4-decadienoic acid isobutylamide. All structures were established by spectroscopic evidence. From the new phenylpropenes, named evofolin-C-acetate, colenemol, colenemal, prenycol acetate, dehydroprenycol acetate, precolpuchol, colpuchol and colpuchol acetate, the dihydroxylated precolpuchol displayed the strongest antifungal and antibacterial activity against *Cladosporium herbarum* and *Staphylococcus aureus*, respectively. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Coleonema pulchellum Williams, a shrubby member of the Rutaceae–Rutoideae (tribe Diosmeae), originates from the Cape province (South Africa). Although this species has been cultivated in many botanical gardens for a long time it has often been confused with *C. pulchrum* Hook. and was only properly described as late as 1981 [1]. Until now only one chemical investigation describes the volatile compounds of the leaves of *C. pulchellum*, namely some phenylpropanoids and terpenoids [2].

In the course of our current screening program for biologically active substances of the Rutaceae, we investigated the lipophilic root extract of *C. pulchellum*. As a result, eight new phenylpropenes were detected besides the already known lignans (±)-sesamin and (±)-prenylpiperitol, the alkamide decadienoic acid isobutylamide, the diterpene (−)-pimara-9(11),15-dien-19-oic acid (**10**), and the phenylpropene evofolin-C (**1**), recently described for *Tetradium glabrifolium* (Champ. ex Benth.) Hartley, another member of the Rutaceae [3]. The new compounds were designated as evofolin-C-acetate (**2**), colenemol (**3**), colenemal (**4**), prenycol acetate (**5**), dehydroprenycol acetate (**6**), precolpuchol (**7**), colpuchol (**8**), and colpulchol acetate (**9**). Here we report the isolation and identification of these compounds as well as on their antifungal and antibacterial properties.

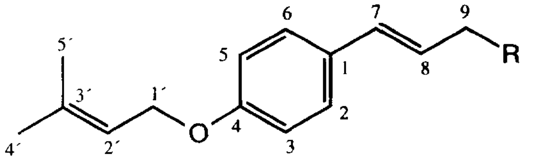
RESULTS AND DISCUSSION

RP-HPLC analyses combined with diode array detection of the chloroform phase of the methanolic root extract revealed a series of compounds with similar UV spectra with only one distinct maximum typical for simple aromatic chromophores. Nearly identical spectra were found for compounds **1**–**3**, **5** and **6**, with maxima (MeOH) between 261 and 267 nm, whereas that of **4** shows a shift towards 321 nm. Compounds **7**–**9** deviate by somewhat different spectra indicating additional chromophoric systems. In the IR spectra the strong signals between 1742 and 1744 cm^{-1} as well as between 1224 and 1228 cm^{-1} of compounds **2**, **5**, **6**, and **9** are typical for ester groups. Compound **4** shows a strong absorption at 1682 cm^{-1} and a characteristic signal at 2732 cm^{-1} indicative of an aldehyde. Stretching vibrations at 3616 cm^{-1} for **1**, **3** and **8** and 3678 cm^{-1} for **7**, indicate OH-groups.

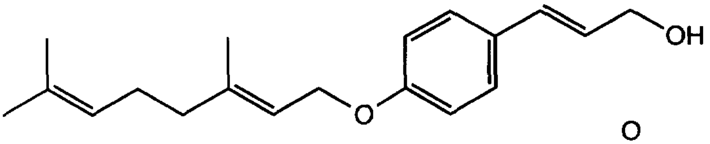
The ^{1}H NMR spectra of **1**–**4** show the characteristic pattern of 1,4-disubstituted benzenes with two second order doublets ($J = 8.7$ Hz, 2H) at approximately $\delta = 7.30$ and 6.90. The chemical shifts of the aromatic protons indicate an oxy-substituent in the *para*-position to an alkyl group. In **1** and **2** the alkyloxy sub-

---

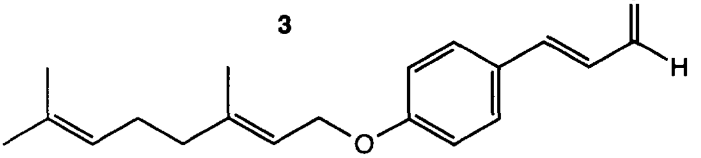
† Author to whom correspondence should be addressed.



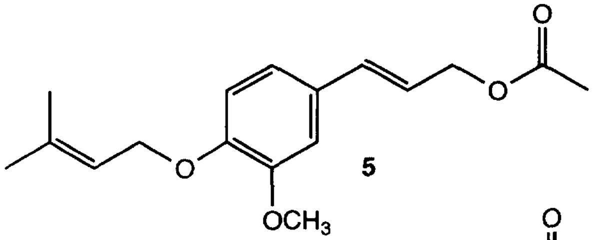
1: R = OH
2: R = OAc



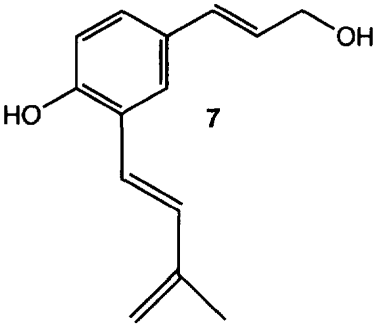
3



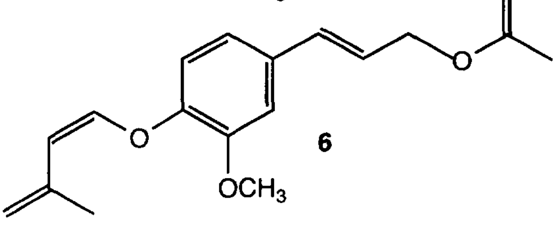
4



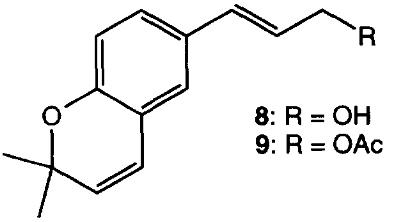
5



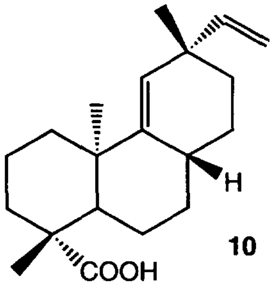
7



6



8: R = OH
9: R = OAc



10

stituents can be identified as prenyloxy groups by means of characteristic ^{1}H and ^{13}C data (see Experimental). The second substituent in **1** is a 3-hydroxy-1-propenyl moiety, with the typical pattern of an ABC system with resonances at $\delta = 6.51$ (*d*, 1H), 6.21 (*dt*, 1H), and 4.32 (*d*, 2H) for $-CH{=}CH-CH_2OH$ and the large olefinic coupling constant of $J = 15.8$ Hz is characteristic of a *trans* configurated double bond. The proposed structure of **1** is supported by the MS data, with a molecular ion of $m/z = 218$ (18%) and a base peak of $m/z = 150$ indicating the loss of a prenyl group. Compound **1**, therefore, was identified as the already known evofolin-C [3]. In the ^{1}H NMR spectrum of **2** the only difference compared with **1** is the appearance of a singlet at $\delta = 2.09$ (3H), which is typical for an acetyloxy ester group. The presence of the ester moiety is also confirmed by the characteristic ^{13}C-resonances at $\delta = 170.9$ for the carbonyl-C and at $\delta = 21$ for the ester methyl group. The mass spectrum of **2** shows a molecular ion of $m/z = 260$ (16%), a base peak at $m/z = 192$ (M after loss of prenyl), and the typical fragmentation pattern of an acetate with fragment ions of $m/z = 201$ [$M^+ - 59$, 2%] and $m/z = 43$ (40%). All spectroscopic data agree with the structure of **2**, namely evofolin-C-acetate.

The mass spectrum of the more unpolar alcohol **3**, named colenemol, was characterized by a molecular ion of $m/z = 286$ (13.6%) and the same base peak as for evofolin-C (**1**) at $m/z = 150$. Hence, **3** has the same basic structure as **1**, but the prenyloxy group is replaced by a longer side chain. In the ^{13}C NMR spectrum this substituent could be determined as a C-10 moiety with resonances of three methyl groups ($\delta = 16.7$, 17.7 and 25.7), three methylene groups (one of them bearing an oxygen function; $\delta = 26.3$, 39.5 and 64.9) and four olefinic carbons (two doublets at $\delta = 119.5$ and 123.8, and two singlets at $\delta = 141.2$ and 131.8). In combination with the resonances of the protons in the ^1H NMR spectrum this C-10 side chain could be clearly identified as a simple geranyloxy group. The three methyl groups appeared as broad singlets at $\delta = 1.60$, 1.68 and 1.73, indicating that they are linked to the two quaternary olefinic carbons. The ^1H NMR coupling pattern and the chemical shifts of the remaining signals agree with the OCH_2–$CH{=}C$– and the CH_2–CH_2–$CH{=}C$– subunit of the geranyloxy moiety. Colenemol **3** therefore represents the geranyl analogue to evofolin-C (**1**).

In accordance with IR- and UV-data, **4** could be clearly identified as the aldehyde of the alcohol **3** and was consequently named colenemal. Compared with **3**, colenemal **4** is less polar and has a molecular ion of $m/z = 284$, which is two units less than the molecular weight of colenemol (**3**). The only difference in the ^1H NMR spectra between these two compounds is the change of the coupling pattern as well as the shift values of the propene moiety. The presence of an aldehyde function is also clearly indicated by a doublet at $\delta = 9.65$ ($J = 7.8$ Hz), which is part of the ABC system of the propene moiety (see Experimental).

While all phenylpropenes described above (**1**–**4**) were shown to be 1,4-disubstituted aromatic compounds, the aromatic proton coupling patterns of the remaining compounds (**5**–**9**), reveal the existence of 1,3,4-trisubstituted benzenes (see Experimental). In accordance with the typical ester signals in the IR spectra of **5** and **6**, the ^1H NMR spectra of these two compounds clearly show resonances of acetate groups at $\delta = 2.10$ (*s*, 3H). Together with the well known signals of the propenol group (see **1**–**3**), this indicates the presence of an 3-acetoxy-1-propenyl moiety as already shown for evofolin-C-acetate **2**. Furthermore, in both compounds one aromatic position is occupied by a methoxy group ($\delta = 3.88$, *s*, 3H). Compound **5**, designated as prenycol acetate (=*O*-prenylconiferylacetate), deviates from **6** by only one different substituent of the aromatic ring. While **5** shows the characteristic resonances of a prenyloxy moiety, the ^1H NMR shifts of the phenylpropene **6** revealed a conjugated dehydroprenyloxy rest with a *cis* configurated double bond ($J = 6.9$ Hz) and a terminal methylene group. The positions of the substituents in the aromatic system were identified by NOE difference measurements: for prenycol acetate (**5**) irradiation of the methoxy protons at $\delta = 3.88$ showed an enhancement of the *meta*-coupling proton at $\delta = 6.93$ and irradiation at $\delta = 4.58$ (O-CH_2 group of the prenyloxy side chain) resulted in a strong positive effect of the *ortho*-coupling proton at $\delta = 6.82$. All these data are only compatible with the structure of prenycol acetate (**5**). On the basis of NOE difference experiments the same substitution pattern could also be assigned to **6**, named dehydroprenycol acetate. Moreover, a further NOE experiment enabled us to distinguish between the two vinyl protons of the dehydroprenyloxy moiety of **6**. Irradiation of 2′-H ($\delta = 5.28$) showed a strong signal enhancement at $\delta = 5.07$ (4′-H), proving that this vinyl proton is *cis* to 2′-H and therefore *trans* to the methyl group. This was also confirmed by the observed long range coupling ($J < 1$ Hz) of 2′-H with the second terminal methylene proton at $\delta = 4.87$ due to the optimal torsional angle resulting in a *W*-coupling. As a consequence, the conformation of the C-2′–C-3′-bond is *s-trans*.

The mass spectra of compounds **7** and **8** are characterized by the same molecular ion peak and base peak of $m/z = 216$ and $m/z = 201$ (M^+–CH_3), respectively. Besides the characteristic coupling pattern of a 1,3,4-trisubstituted aromatic system and the well known propenol moiety, the low-field region in the ^1H NMR spectrum of **7** reveals signals of two *trans*-coupled protons at $\delta = 6.91$ and 6.76, and a terminal methylene group ($=CH_2$, $\delta = 5.13$ and 5.10, both as broad singlets). Considering also the additional methyl resonance at $\delta = 2.00$, the hemiterpene unit was identified as a 3-methyl-1,3-butadienyl residue. NOE experiments proved this unit to be linked at position 3 of the benzene system (irradiation of 2-H shows strong signal enhancements of 7-H, 8-H, and 1′-H). Further, positive NOEs were observed by irradiation of 1′-H (a strong effect on one of the terminal methylene protons at $\delta = 5.13$) and by irradiation of the methyl-protons ($\delta = 2.00$, signal enhancement of 2′-H). These results allowed interesting conclusions concerning the conformation of the side chain. In contrast to the favoured *s-trans* conformation of the dehydroprenyloxy substituent of **6**, the dehydroprenyl moiety of **7**, named precolpuchol, showed a *s-cis* arrangement. An additional broad singlet of one H at $\delta = 5.44$ indicates a phenolic hydroxyl group at position 4. Compounds **8** and **9** were proved to be the corresponding 2,2-dimethylpyrano derivatives of precolpuchol **7** with the typical pyrano resonances at $\delta = 6.31$, 5.62 (both *d*, $J = 9.8$ Hz), and 1.43 (*s*, 6H). Compared to the alcohol **8**, named colpuchol, **9** shows an additional singlet at $\delta = 2.09$ and was therefore identified as the corresponding acetate, named colpuchol acetate (**9**). This assumption was confirmed by the typical MS fragmentation pattern with a base peak of $m/z = 215$ [M^+–$COCH_3$] and fragment ions of $m/z = 199$ [15%, M^+–$OCOCH_3$] and of $m/z = 43$ (20%).

In addition to this series of phenylpropenes the root extract of *C. pulchellum* also contains large amounts of the diterpene (−)-pimara-9(11),15-dien-19-oic acid (**10**), already identified from *Acanthopanax koreanum* Nakai [4], as well as the well known alkamide 2,4-decadienoic acid isobutylamide [5]. Both structures were confirmed by spectroscopic analyses, whereas the two tetrahydrofurofuranlignans (±)-sesamin and (±)-prenylpiperitol were identified by HPLC-UV-comparisons with authentic samples.

The accumulation of C_6–C_3 derivatives obviously represent a characteristic biogenetic trend of the tribe Diosmeae, where at least coumarins are known to be widely distributed [6, 9]. Within the genus *Coleonema* only the aerial parts have been investigated so far, showing a series of prenylated coumarins in *C. album* Bartl. & Wendl. [7], *C. aspalathoides* A. Juss. and *C. calycinum* (Steud.) Williams [8], as well as the already mentioned volatile constituents in *C. pulchellum* [2]. By means of comparative HPLC-analyses, we now also find small amounts of coumarins in the aerial parts of *C. pulchellum*, together with the phenylpropenes described above. Although we could not detect any coumarin in the root extract of that species, the accumulation of prenylated phenylpropenes and lignans also fits into this common biosynthetic scheme. However, for chemosystematic interpretations it should be taken into consideration that prenylated phenylpropanoids are widely distributed within the whole Rutaceae and, hence are of limited systematic value. Apart from the already mentioned *Tetradium glabrifolium* [3], *Zanthoxylum* species were shown to be a rich source of different phenylpropene derivatives [10, 11].

The newly described phenylpropenes **1–8** and the diterpene **10** were tested for antifungal and antibacterial properties. Using bioautography on TLC plates, the fungitoxic activity was determined with spore suspensions of our test fungus *Cladosporium herbarum* [12]. Precolpuchol (**7**) displayed the strongest inhibition upon spore germination and mycelial growth, whereas the other derivatives show either moderate activity, e.g. in evofolin-C (**1**), or no effects at all. In a serial dilution precolpuchol (**7**) caused a clearly visible inhibition zone, even at the lowest concentration at 5 μg. This antifungal activity is comparable with that of the previously described sulphur-containing amides from *Glycosmis* species (Rutaceae–Aurantioideae) [12, 13]. TLC bioassays were also used to detect the antibacterial properties [14] of the isolated compounds. Again, precolpuchol (**7**) showed the highest activity against *Staphylococcus aureus* at a concentration as low as 0.5 μg. Moreover, colenemal (**4**) and the diterpene **10** also exhibited antibacterial activity with concentrations of 5 μg. The strong bioactivity of **7** might be explained by the *s-cis* conformation of the two double bonds, which could participate in a Diels–Alder reaction with other π-systems. Beyond that, another mode of action could also be deduced from the reactivity of the phenolic hydroxyl group.

EXPERIMENTAL

General. NMR: Bruker AC-250W (250 MHz), $CDCl_3$. MS: Varian MAT-CH7. IR: Perkin–Elmer 16 PC FT-IR. HPLC: UV diode-array detection: 230 nm, column 290 × 4 mm (Spherisorb ODS, 5 μm), mobile phase MeOH (gradient 60–100%) in aq. buffer (*o*-phosphoric acid 0.015 M, tetrabutylammonium hydroxide 0.0015 M, pH 3), flow rate 1 ml min^{-1}.

Plant material. Coleonema pulchellum Williams was grown from seeds received from the Botanical Garden of Coimbra, Portugal. The plants were cultivated under field conditions in the Botanical Garden of the University of Vienna (HBV) and were transferred into the greenhouse during winter. Voucher specimens are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

Extraction and isolation. Air-dried roots (115 g) were extracted with MeOH at room temp. for 3 days, filtered and concd. The $CHCl_3$ fr. from the aq. soln. was evapd to dryness (1300 mg) under red pres. and roughly sepd by CC (Merck silica gel 60, 35–70 mesh). Further sepn by prep. MPLC with 5% EtOAc in petrol (400 × 38 mm column, Merck LiChroprep Si 60, 25–40 μm, UV detection, 254 nm) afforded 96 mg colenemal (**4**), 3 mg prenycol acetate (**5**), 3 mg dehydroprenycol acetate (**6**), 1 mg colpuchol acetate (**9**) and 150 mg evofolin-C acetate (**2**) together with 100 mg of (−)-pimara-9(11),15-diene-19 oic-acid (**10**). **2** and **10** were further purified by TLC using CH_2Cl_2–Et_2O (95:5) as eluent. 10% EtOAc yielded 9 mg colenemol (**3**), 21 mg sesamin and a mixt. of three compounds which could further sepd by Sephadex LH-20 with MeOH to give 4 mg evofolin-C (**1**), 3 mg prenylpiperitol and 13 mg 2,4-decadienoic acid isobutylamide. 30% EtOAc afforded 25 mg precolpuchol (**7**) and 1 mg colpuchol (**8**).

Evofolin-C {3-[4-(4-*methyl*-2-*butenyloxy*)-*phenyl*]-2-*propenol*} (**1**). Oil. UV λ^{Et_2O} nm: 218, 263, 294 (sh), 306 (sh). IR ν^{CCl_4} cm^{-1}: 3616 w, 3492 vw, 3042 w, 2974 m, 2928 m, 2860 m, 1606 m, 1576 w, 1510 s, 1464 w, 1448 w, 1382 m, 1302 m, 1240 s, 1174 m, 1112 w, 1088 w, 1006 m, 966 m, 842 w. ^1H NMR ($CDCl_3$): δ 7.31 (*d*, 2H, $J = 8.7$ Hz, 2- and 6-H_{Ar}), 6.87 (*d*, 2H, $J = 8.7$ Hz, 3- and 5-H_{Ar}), 6.56 (*br d*, 1H, $J = 15.9$ Hz, 7-H), 6.23 (*dt*, 1H, $J = 15.9$ and 5.9 Hz, 8-H), 5.49 (*tsept*, 1H, $J = 6.7$ and 1.4 Hz, 2′-H), 4.51 (*d*, 2H, $J = 6.7$ Hz, 1′-H), 4.29 (*dd*, 2H, $J = 5.9$ and <1 Hz, 9-H), 1.80 (*br s*, 3H, 5′-H), 1.75 (*br s*, 3H, 4′-H). MS (70 eV, 40°) *m*/*z* (rel. int.): 218 (18) [M^+, $C_{14}H_{18}O_2$], 150 (100), 131 (11), 121 (10), 107 (99), 94 (76), 91 (9), 77 (13), 69 (43), 55 (8).

Evofolin-C acetate [3-{4-(3-*methyl*-2-*butenyloxy*)-*phenyl*}-2-*propenyl acetate*] (**2**). Oil. UV λ^{Et_2O} nm: 218, 263, 294 (sh), 306 (sh). IR ν^{CCl_4} cm^{-1}: 3034 w, 2978 w, 2934 m, 2874 w, 1742 s, 1606 m, 1510 s, 1446 w, 1380 m, 1362 m, 1228 s, 1174 m, 1022 m, 966 m, 842 w. ^1H NMR ($CDCl_3$): δ 7.32 (*d*, 2H, $J = 8.7$ Hz, 2- and 6-H_{Ar}), 6.86 (*d*, 2H, $J = 8.7$ Hz, 3- and 5-H_{Ar}), 6.60 (*br d*, 1H, $J = 15.9$ Hz, 7-H), 6.14 (*dt*, 1H, $J = 15.9$ and 6.6 Hz, 8-H), 5.49 (*tsept*, 1H, $J = 6.7$ and 1.3 Hz, 2′-H), 4.70 (*dd*, 2H, $J = 6.7$ and 1.1 Hz, 9-H), 4.51 (*d*, 2H, $J = 6.7$ Hz, 1′-H), 2.09 (*s*, 3H, Ac-H_3), 1.79 (*s*, 3H, 5′-H), 1.74 (*s*, 3H, 4′-H). ^{13}C NMR ($CDCl_3$, 62.9

MHz): δ 170.9 (*s*, ester CO), 158.9 (*s*, 4′-C), 138.2 (*s*, 3′-C), 134.1 (*d*, 7-C), 128.8 (*s*, 1-C), 127.8 (*d*, 2-C and 6-C), 120.7 (*d*, 8-C), 119.6 (*d*, 2′-C), 114.8 (*d*, 3-C and 5-C), 65.3 (*t*, 9-C), 64.8 (*t*, 1′-C), 25.8 (*q*, 4′-C), 21.0 (*q*, ester CH$_3$), 18.2 (*q*, 5′-C). MS (70 eV, 40°) *m/z* (rel. int.): 260 (16) [M$^+$, $C_{16}H_{20}O_3$], 192 (100), 149 (96), 133 (49), 121 (33), 107 (21), 103 (11), 94 (21), 77 (11), 69 (62), 43 (40).

Colenemol {3-[4-*dimethyl*-2,6-*octadienyloxy*)-*phenyl*]-2-*propenol*} (**3**). Oil. UV λ^{Et_2O} nm: 218, 263, 293 (sh), 305 (sh). IR ν^{CCl_4} cm^{-1}: 3616 w, 3492 vw, 2978 m, 2928 m, 2866 m, 1608 w, 1510 m, 1444 w, 1382 m, 1350 w, 1298 w, 1240 m, 1174 m, 1152 w, 1120 s, 1006 w, 966 w, 844 w. ^{1}H NMR (CDCl$_3$: δ 7.31 (*d*, 2H, *J* = 8.7 Hz, 2- and 6-H$_{Ar}$), 6.87 (*d*, 2H, *J* = 8.7 Hz, 3- and 5-H$_{Ar}$), 6.55 (*br d*, 1H, *J* = 15.9 Hz, 7-H), 6.23 (*dt*, 1H, *J* = 15.9 and 5.9 Hz, 8-H), 5.48 (*tq*, 1H, *J* = 6.7 and <1 Hz, 2′-H), 5.09 (*m*, 1H, 6′-H), 4.54 (*d*, 2H, *J* = 6.5 Hz, 1′-H), 4.30 (*dd*, 2H, *J* = 5.9 and 1.4 Hz, 9-H), 2.10 (*m*, 4H, 4′-H$_2$ and 5′-H$_2$), 1.73 (*s*, 3H, 10′-H), 1.68 (*s*, 3H, 9′-H), 1.60 (*s*, 3H, 8′-H). ^{13}C NMR (CDCl$_3$ 62.9 MHz): δ 158.7 (*s*, 4-C), 141.2 (*s*, 3′-C), 131.8 (*s*, 7′-C), 131.1 (*d*, 7-C), 129.3 (*s*, 1-C), 127.6 (*d*, 2-C and 6-C), 126.2 (*d*, 8-C), 123.8 (*d*, 6′-C), 119.5 (*d*, 2′-C), 114.8 (*d*, 3-C and 5-C), 64.9 (*t*, 1′-C), 64.0 (*t*, 9-C), 39.5 (*t*, 4′-C), 26.3 (*t*, 5′-C), 25.7 (*q*, 9′-C), 17.7 (*q*, 10′-C), 16.7 (*q*, 8′-C). MS (70 eV, 110°) *m/z* (rel. int.): 286 (14) [M$^+$, $C_{19}H_{26}O_2$], 150 (100), 121 (31), 107 (93), 94 (84), 81 (76), 69 (96), 55 (36).

Colenemal {3-[4-(3,7-*dimethyl*-2,6-*octadienyloxy*)-*phenyl*]-2-*propenal*} (**4**). Crystals, mp 36–38°. UV λ^{Et_2O} nm: 218, 232, 303 (sh), 314. IR ν^{CCl_4} cm^{-1}: 3038 w, 2968 w, 2928 m, 2732 w, 1682 s, 1624 m, 1602 s, 1572 w, 1510 s, 1452 w, 1426 w, 1378 w, 1306 w, 1296 w, 1248 m, 1172 m, 1125 s, 1002 m, 970 m, 850 w. ^{1}H NMR (CDCl$_3$): δ 9.65 (*d*, 1H, *J* = 7.8 Hz, 9-H), 7.51 (*d*, 2H, *J* = 8.7 Hz, 2- and 6-H$_{Ar}$), 7.42 (*d*, 1H, *J* = 15.8 Hz, 7-H), 6.95 (*d*, 2H, *J* = 8.7 Hz, 3- and 5-H$_{Ar}$), 6.60 (*dd*, 1H, *J* = 15.8 and 7.8 Hz, 8-H), 5.47 (*br t*, 1H, *J* = 6.5 Hz, 2′-H), 5.08 (*m*, 1H, 6′-H), 4.59 (*d*, 2H, *J* = 6.5 Hz, 1′-H), 2.10 (*m*, 4H, 4′-H$_2$ and 5′-H$_2$), 1.75 (*s*, 3H, 10′-H), 1.67 (*s*, 3H, 9′-H), 1.60 (*s*, 3H, 8′-H). ^{13}C NMR (CDCl$_3$, 62.9 MHz): δ 193.6 (*d*, 9-C), 161.5 (*s*, 4-C), 152.7 (*d*, 7-C), 141.8 (*s*, 3′-C), 131.9 (*s*, 7′-C), 130.3 (*d*, 2-C and 6-C), 126.6 (*s*, 1-C), 126.4 (*d*, 8-C), 123.6 (*d*, 6′-C), 118.8 (*d*, 2′-C), 115.2 (*d*, 3-C and 5-C), 65.1 (*t*, 1′-C), 39.5 (*t*, 4′-C), 26.2 (*t*, 5′-C), 25.6 (*q*, 9′-C), 17.6 (*q*, 10′-C), 16.6 (*q*, 8′-C). MS (70 eV, 130°) *m/z* (rel. int.): 284 (3) [M$^+$, $C_{19}H_{24}O_2$], 205 (4), 148 (83), 136 (75), 131 (38), 120 (35), 107 (17), 93 (82), 81 (82), 69 (100), 55 (37).

Prenycol acetate {3-[3-*methoxy*-4-(3-*methyl*-2-*butenyloxy*)-*phenyl*]-2-*propenyl acetate*} (**5**). Oil. UV λ^{Et_2O} nm: 219, 268, 295 (sh). IR ν^{CCl_4} cm^{-1}: 2960 m, 2928 s, 2856 m, 1742 s, 1602 w, 1510 s, 1464 m, 1418 w, 1378 w, 1264 m, 1224 s, 1162 w, 1140 m, 1024 m, 964 m, 856 w. ^{1}H NMR (CDCl$_3$): δ 6.93 (*d*, 1H, *J* = 2.1 Hz, 2-H$_{Ar}$), 6.90 (*dd*, 1H, *J* = 8.2 and 2.1 Hz, 6-H$_{Ar}$), 6.82 (*d*, 1H, *J* = 8.2 Hz, 5-H$_{Ar}$), 6.59 (*br d*, 1H, *J* = 15.8 Hz, 7-H), 6.16 (*dt*, 1H, *J* = 15.8 and 6.6 Hz, 8-H), 5.51 (*tsept*, 1H, *J* = 6.8 and <1 Hz, 2′-H), 4.71 (*dd*, 2H, *J* = 6.7 and 1.3 Hz, 9-H), 4.58 (*d*, 2H, *J* = 6.8 Hz, 1′-H), 3.88 (*s*, 3H, –OCH$_3$), 2.09 (*s*, 3H, Ac-H$_3$), 1.77 (*br s*, 3H, 5′-H), 1.73 (*br s*, 3H, 4′-H). MS (70 eV, 90°) *m/z* (rel. int.): 290 (5) [M$^+$, $C_{17}H_{22}O_4$], 257 (10), 222 (100), 179 (59), 163 (20), 151 (17), 147 (16), 131 (40), 124 (18), 119 (28), 105 (16), 93 (12), 91 (25), 81 (14), 69 (45).

Dehydroprenycol acetate {3-[3-*methoxy*-4-(3-*methyl*-1,3-*butadienyloxy*)-*phenyl*]-2-*propenyl acetate*} (**6**). Oil. UV λ^{Et_2O} nm: 219, 269, 298 (sh). IR ν^{CCl_4} cm^{-1}: 2958 m, 2928 m, 2854 w, 1744 s, 1710 w, 1650 m, 1598 w, 1508 s, 1464 m, 1418 w, 1378 w, 1272 s, 1224 s, 1160 w, 1134 w, 1066 m, 1040 w, 1024 w, 964 m, 880 w. ^{1}H NMR (CDCl$_3$): δ 6.90–7.00 (*m*, 3H, 2-H$_{Ar}$, 5-H$_{Ar}$, 6-H$_{Ar}$), 6.60 (*br d*, 1H, *J* = 15.8 Hz, 7-H), 6.24 (*d*, 1H, *J* = 6.9 Hz, 1′-H), 6.20 (*dt*, 1H, *J* = 15.8 and 6.5 Hz, 8-H), 5.28 (*dd*, 1H, *J* = 6.9 and 1 Hz, 2′-H), 5.07 (*dq*, 1H, *J* = 2.4 and <1 Hz, 4′-H$_a$), 4.87 (*ddq*, 1H, *J* = 2.4, 1.2 and <1 Hz, 4′-H$_b$), 4.72 (*dd*, 2H, *J* = 6.7 Hz, 9-H), 3.88 (*s*, 3H, OCH$_3$), 2.10 (*s*, 3H, Ac-H$_3$), 2.09 (*br s*, 3H, 5′-H). MS (70 eV, 90°) *m/z* (rel. int.): 288 (40) [M$^+$, $C_{17}H_{20}O_4$], 228 (37), 213 (52), 197 (19), 185 (18), 164 (25), 146 (31), 131 (25), 115 (20), 103 (19), 97 (10), 91 (18), 82 (17), 71 (15), 69 (24), 55 (28), 43 (100).

Precolpuchol {3-[4-*hydroxy*-3-(3-*methyl*-1,3-*butadienyl*)-*phenyl*]-2-*propenol*} (**7**). Oil. UV λ^{Et_2O} nm: 220, 269, 327. IR ν^{CHCl_3} cm^{-1}: 3678 w, 3596 m, 3314 br, 2928 s, 2856 m, 1708 w, 1602 m, 1506 m, 1452 m, 1378 m, 1316 w, 1266 s, 1102 m, 1004 w, 968 s, 854 w. ^{1}H NMR (CDCl$_3$): δ 7.47 (*d*, 1H, *J* = 2.1 Hz, 2-H$_{Ar}$), 7.17 (*dd*, 1H, *J* = 8.3 and 2.1 Hz, 6-H$_{Ar}$), 6.91 (*d*, 1H, *J* = 16.2 Hz, 1′-H), 6.76 (*d*, 1H, *J* = 16.2 Hz, 2′-H), 6.73 (*d*, 1H, *J* = 8.3 Hz, 5-H$_{Ar}$), 6.56 (*br d*, 1H, *J* = 15.9 Hz, 7-H), 6.27 (*dt*, 1H, *J* = 15.9 and 5.9 Hz, 8-H), 5.13 (*br s*, 1H, 4′-H$_a$), 5.10 13 (*br s*, 1H, 4′-H$_b$), 4.32 (*dd*, 2H, *J* = 5.9 and <1 Hz, 9-H), 2.00 (*s*, 3H, 5′-H). MS (70 eV, >300°) *m/z* (rel. int.): 216 (30) [M$^+$, $C_{14}H_{16}O_2$], 201 (100), 183 (19), 171 (27), 159 (35), 141 (20), 128 (26), 115 (30), 91 (28), 69 (28), 57 (62), 43 (52).

Colpuchol {3-(2,2-*dimethyl*-1,2-*benzopyran*-6-*yl*)-2-*propenol*} (**8**). Oil. UV λ^{Et_2O} nm: 221 (sh), 246, 253 (sh), 278, 326. IR ν^{CCl_4} cm^{-1}: 3616 w, 3042 w, 2976 m, 2926 m, 2868 w, 1638 w, 1608 w, 1490 s, 1464 w, 1370 m, 1362 m, 1266 s, 1212 m, 1154 m, 1128 m, 1106 m, 1088 m, 1008 m, 964 s, 904 w, 890 w, 852 w. ^{1}H NMR (CDCl$_3$): δ 7.14 (*dd*, 1H, *J* = 8.3 and 2.1 Hz, 6-H$_{Ar}$), 7.02 (*d*, 1H, *J* = 2.1 Hz, 2-H$_{Ar}$), 6.72 (*d*, 1H, *J* = 8.3 Hz, 5-H$_{Ar}$), 6.51 (*br d*, 1H, *J* = 15.8 Hz, 7-H), 6.31 (*d*, 1H, *J* = 9.8 Hz, 1′-H), 6.21 (*dt*, 1H, *J* = 15.8 and 5.9 Hz, 8-H), 5.62 (*d*, 1H, *J* = 9.8 Hz, 2′-H), 4.72 (*dd*, 2H, *J* = 6.5 and 1 Hz, 9-H), 1.43 (*s*, 6H, 4′-H$_3$ and 5′-H$_3$). MS (70 eV, 100°), *m/z* (rel. int.): 216 (35) [M$^+$, $C_{14}H_{16}O_2$], 201 (100), 171 (19), 149 (33), 121 (17), 115 (11), 91 (21), 81 (24), 69 (13).

Colpuchol acetate {3-(2,2-*dimethyl*-1,2-*benzopyran*-6-*yl*)-2-*propenyl acetate*} (**9**). Oil. UV λ^{Et_2O} nm: 219 (sh), 248, 254, 278, 327. IR ν^{CCl_4} cm^{-1}: 3042 w, 2958 m, 2928 s, 2854 m, 1742 s, 1638 w, 1606 w, 1488 s, 1464 m, 1370 m, 1362 m, 1266 s, 1228 s, 1212 m, 1152 m, 1128 m, 1108 m, 964 m, 908 w, 890 w, 860 w. ^{1}H NMR ($CDCl_3$): δ 7.14 (*dd*, 1H, $J = 8.4$ and 2 Hz, 6-H_{Ar}), 7.02 (*d*, 1H, $J = 2$ Hz, 2-H_{Ar}), 6.72 (*d*, 1H, $J = 8.4$ Hz, 5-H_{Ar}), 6.50 (*br d*, 1H, $J = 15.9$ Hz, 7-H), 6.30 (*d*, 1H, $J = 9.8$ Hz, 1′-H), 6.12 (*dt*, 1H, $J = 15.9$ and 6.6 Hz, 8-H), 5.62 (*d*, 1H, $J = 9.8$ Hz, 2′-H), 4.69 (*dd*, 2H, $J = 6.6$ and <1 Hz, 9-H), 2.09 (*s*, 3H, Ac-H_3), 1.43 (*s*, 6H, 4′-H_3 and 5′-H_3). MS (70 eV, 40°) *m/z* (rel. int.): 258 (21) [M^+, $C_{16}H_{18}O_3$], 243 (90), 230 (32), 215 (100), 199 (15), 183 (32), 169 (16), 149 (11), 128 (9), 115 (12), 97 (10), 85 (12), 69 (14), 57 (23), 55 (16), 43 (20).

Acknowledgement—Financial support by the Austrian National Committee for the Intergovernmental Programme 'Man and Biosphere' is gratefully acknowledged.

REFERENCES

1. Williams, I., *Journal of South African Botany*, 1981, **47**, 63.
2. Brophy, J. J. and Lassak, E. U., *Flavour and Fragrance Journal*, 1986, **1**, 155.
3. Wu, T.-S., Yeh, J.-H. and Wu, P.-L., *Journal of the Chinese Chemical Society*, 1995, **42**, 107.
4. Kim, Y. H., Chung, B. S. and Sankawa, U., *Journal of Natural Products*, 1988, **51**, 1080.
5. Greger, H., in *Chemistry and Biology of Naturally-Occurring Acetylenes and Related Compounds* (*NOARC*), ed. J. Lam, H. Breteler, T. Arnason and L. Hansen. Elsevier, Amsterdam, 1988, p. 159.
6. Gray, A. I., in *Chemistry and Chemical Taxonomy of the Rutales*, ed. P. G. Waterman and M. F. Grundon. Academic Press, London, 1983, p. 97.
7. Gray, A. I., *Phytochemistry*, 1981, **20**, 1711.
8. Gray, A. I., Meegan, C. J. and O'Callaghan, N. B., *Phytochemistry*, 1987, **26**, 257.
9. Campbell, W. E., Majal, T. and Bean, P. A., *Phytochemistry*, 1986, **25**, 655.
10. Shibuya, H., Takeda, Y., Zhang, R., Tong, R.-X. and Kitagawa, I., *Chemical and Pharmaceutical Bulletins*, 1992, **40**, 2325.
11. Ishii, H., Ishikawa, T., Tohojoh, T., Murakami, K., Kawanabe, E., Lu, S.-T. and Chen, I.-S., *Journal of the Chemical Society, Perkin Transactions*, 1982, **1**, 2051.
12. Greger, H., Zechner, G., Hofer, O., Hadacek, F. and Wurz, G., *Phytochemistry*, 1993, **34**, 175.
13. Greger, H., Zechner, G., Hofer, O. and Vajrodaya, S., *Journal of Natural Products*, 1996, **59**, 1163.
14. Cole, M. D., *Biochemical Systematics and Ecology*, 1994, **8**, 837.