REFERENCES

1. Fischer, F. C., Jasperse, P. H., Karlsen, J. and Baerheim Svendsen, A. (1974) *Phytochemistry* **13**, 2334.
2. Steck, W. and Wetter, L. R. (1974) *Phytochemistry* **13**, 1925.
3. Fischer, F. C. and Doorne, H. van. To be published.
4. Kamat, V. S., Audichya, T. D., Trivedi, G. K. and Bhattacharyya, S. C. (1975) *J. Chem. Soc. Perkin I*, 204.
5. Pedersen, B. and Karlsen, J. (1976) *Acta Crystallog.* (In press).

Phytochemistry, 1976, Vol. 15, pp. 1080–1081. Pergamon Press. Printed in England.

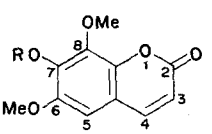
PUBERULIN, A NEW PRENYLOXY-COUMARIN FROM *AGATHOSMA PUBERULA*

NATHAN FINKELSTEIN and DOUGLAS E. A. RIVETT
Department of Chemistry, Rhodes University, Grahamstown 6140, Republic of South Africa

(*Received* 26 *October* 1975)

Key Word Index—*Agathosma puberula*; Rutaceae; 6,8-dimethoxy-7-prenyloxycoumarin.

Recently, we have investigated the essential oils of several *Agathosma* species and shown the presence of the biogenetically intriguing thioester, *S*-prenyl thioisobutyrate in *A. apiculata* G. F. W. Mey, *A. clavisepala* R. A. Dyer and *A. puberula* Fourc. [1]. This report describes the isolation and identification of a novel coumarin, puberulin, (6,8-dimethoxy-7-prenyloxycoumarin) from the eastern Cape Province species *A. puberula* Fourc. Puberulin (1) crystallized as colourless platelets, mp 90–92°, and analysed for $C_{16}H_{18}O_5$ (M^+ 290). It gave a blue fluorescence in UV light (366 nm) and a "false positive" orange spot with Dragendorff's reagent [2]. The UV spectrum is consistent with that of a substituted coumarin having no OH-substituents, since the addition of alkali produced no bathochromic shift. The IR spectrum had a strong absorption band at 1720 cm^{-1} indicative of a coumarinyl lactone. The PMR spectrum of puberulin defined all eighteen protons. The two doublets at δ6.35 and δ7.66 ($J = 9.5$ Hz) are due to H-3 and H-4. The chemical shift of the latter shows that C-5 must contain no oxygen function otherwise it would appear at δ7.8–8.2 [3]. Accordingly, the one proton singlet at δ6.71 is assigned to H-5. The presence of two methoxyl signals at δ4.05 and δ3.91 and a prenyl substituent, typified by a methylene doublet at δ4.66 ($J = 6$ Hz), a coupled olefinic triplet at δ5.60 and two non-equivalent methyl resonances at δ1.73 and δ1.79 confirms that these three alkyl substituents occupy the three vacant positions. The relative positions of these substituents were firmly established as follows.



(**1**) R = $Me_2C{=}CHCH_2$
(**2**) R = H
(**3**) R = Me
(**4**) R = Et
(**5**) R = Ac

Hydrogenation of puberulin (**1**) over Paal catalyst yielded a phenol, $C_{11}H_{10}O_5$, mp 147–148°, which gave a greenish ppt. with $FeCl_3$ and showed ν_{max} 3520 cm^{-1}. The PMR spectrum was very similar to that of puberulin with reference to the pyrone ring doublets (δ6.33 and δ7.68), the singlet due to H-5 (δ6.74) and the two methoxyl signals (δ3.96 and δ4.10). The signals of the prenyl group in puberulin (**1**) had disappeared and had been replaced by a one proton singlet at δ6.33, which in turn, exchanged readily with D_2O. This facile hydrogenolysis of the prenyl to a hydroxyl group shows that the former is originally present as an *O*-prenyl rather than a *C*-prenyl group. The low abundance (3%) of M^+ in the MS of puberulin and the intense fragments at *m/e* 69 (96%; prenyl) and *m/e* 222 (100%; isofraxidin) are consistent with an *O*-prenyl coumarin [4,5]. In spite of several attempts no dihydro-derivative (cf. phellopterin [6]) could be isolated.

All three of the possible 6,7,8-dimethoxyhydroxycoumarins are known, viz. 6,7-dimethoxy-8-hydroxycoumarin (mp 195°) [7], 7,8-dimethoxy-6-hydroxycoumarin 184°) [8] and 6,8-dimethoxy-7-hydroxycoumarin (mp 148–9°) [9]. That the phenol, mp 147–8°, was 6,8-dimethoxy-7-hydroxycoumarin (isofraxidin)(**2**) was proved by conversion to the methyl (**3**) and ethyl (**4**) ethers, the mp's of which agreed with those in the literature [8,9]. The acetate (**5**) is a new derivative of isofraxidin. Finally, prenylation of our phenol afforded a product indistinguishable by IR, TLC and mmp from puberulin (**1**).

Because *S*-prenyl thioisobutyrate was present in *A. puberula*, *A. clavisepala* and *A. apiculata*, it was expected that prenylated coumarins would also occur in the latter two species. However, no puberulin could be detected, by means of TLC, in hexane extracts of *A. apiculata* or *A. clavisepala* (in large scale experiments a trace (0.03%) was subsequently found in *A. clavisepala*) or even in *A. ovata* (Thunb.) Pillans, which contains no thioester [1]. Most of the puberulin in *A. puberula* occurs in the leaves (1.26%) and only a trace (0.08%) is present in the stems and twigs. Also, the mother liquors from puberulin did not contain any other coumarins.

Coumarins having prenyl, geranyl and farnesyl substituents have been found chiefly amongst members of the Rutaceae and Umbelliferae [3]. Puberulin is closely related to certain sesquiterpene ethers of isofraxidin occurring in *Artemisia* [10] and *Anthemis* spp. [4]. Subsequent to our work, Bohlmann *et al.* [11] have reported the isolation of puberulin as an impure oil from *Pteronia ciliata* Thunb. (Compositae) but the structure was not completely elucidated.

EXPERIMENTAL

IR, UV and 60MHz PMR spectra were recorded in $CHCl_3$, EtOH and $CDCl_3$ with TMS as internal reference respectively. MS were determined on a double-focussing instrument (AEI MS 9) at 70eV and mp's on a Kofler block (uncorrected). TLC was done on Si gel and developed with C_6H_{14}–EtOAc (1:1).

Plant material. Agathosma puberula Fourc. was collected on the farm 'Upper Gletwyn' 13 km east of Grahamstown. *Agathosma apiculata* G. F. W. Mey was collected at Port Alfred, *Agathosma ovata* (Thunb.) Pillans 0.5 km beyond the Leather Industries Research Institute, Grahamstown and *Agathosma clavisepala* R. A. Dyer was collected 2 km beyond the farm 'Longford Grange' in the Alexandria district. The plants were authenticated by Mrs. E. Brink of the Albany Museum, Grahamstown, where voucher specimens have been lodged under the following numbers:—*A. puberula* (A1745), *A. apiculata* (A1981), *A. ovata* (A1739) and *A. clavisepala* (A1982). The aerial parts (i.e. stems and leaves severed ± 10 cm from soil level) were air-dried for 6 weeks and comminuted in a Retsch mill (2 mm sieve). Additionally, in the case of *A. puberula*, the leaves were carefully hand-picked and separated from stems and twigs and separately milled.

Extraction. The powdered aerial parts (1.56 kg), stems and twigs (2 kg) and leaves (1.70 kg) of *A. puberula*, aerial parts (1.6 kg) of *A. ovata*, aerial parts (1.6 kg) of *A. apiculata* and aerial parts (2 kg) of *A. clavisepala* were each in turn extracted in a Soxhlet with *n*-hexane (10 l.) for 48 hr. The extracts were concentrated under reduced pressure to approx. 2 l. All the extracts from *A. puberula* crystallized whereas those from the other species did not.

Isolation of puberulin (**1**). Crystalline deposits from the aerial parts (7.8 g, 0.5%), stems and twigs (1.7 g, 0.08%) and leaves (21.4 g, 1.26%) were all purified in the same way. A sample (500 mg) in MeOH (20 ml) and 10% aq. NaOH (10 ml) was heated for 1 min. After 24 hr, the insoluble ppt. was removed and washed with H_2O. The filtrate was extracted with Et_2O (20 ml). MeOH was removed under red. pres. from the clear yellow aq phase and the solution cooled in ice. The soln was carefully acidified and extracted with Et_2O (3 × 20 ml) and dried. Removal of the solvent afforded a yellow oil (470 mg) which was chromatographed on neutral alumina and crystallized from C_6H_6–petrol (40–60°) to yield colourless platelets (120 mg), mp 90–92°; UV λ_{max} (ϵ): 342 (7200), 298 (10400), 228 *sh.* (18000), 210 (35600) nm; IR ν_{max}: 1720, 1610, 1565, 1485, 1460, 1410, 1350, 1290, 1150, 1125, 1085, 1035, 970 cm^{-1}; PMR: δ1.73 (3H, *s*), 1.79 (3H, *s*), 3.91 (3H, *s*), 4.05 (3H, *s*) 4.66 (2H, *d*, *J* 6 Hz), 5.60 (1H, *t*, *J* 6 Hz), 6.35 (1H, *d*, *J* 9.5 Hz), 6.71 (1H, *s*), 7.66 (1H, *d*, *J* 9.5 Hz); MS *m/e* (rel. int.): 290 M^+ (3), 223 (61), 222 (100), 207 (54), 194 (27), 179 (18), 176 (22), 79 (18), 78 (63), 69 (96). (Found: C, 65.93; H, 5.93. $C_{16}H_{18}O_5$ requires: C, 66.19; H, 6.25%).

Hydrogenation of (**1**). A soln of (**1**) (500 mg) in EtOH was hydrogenated over Paal catalyst [12] for 15 min., filtered and concentrated. The residue was dissolved in C_6H_6 and passed through a short column of neutral alumina. Crystallization from C_6H_6 and then from aq EtOH (charcoal) furnished colourless needles (75 mg) of 6,8-dimethoxy-7-hydroxycoumarin (isofraxidin)(**2**), mp 147–148°)(lit[9] mp 148–149°); IR ν_{max}: 3530, 1720, 1610, 1565, 1500, 1460, 1410, 1370, 1310, 1250, 1150, 1115, 1080, 1030, 970 cm^{-1}; PMR: δ3.96 (3H, *s*), 4.10 (3H, *s*), 6.33 (1H, *d*, *J* 9.5 Hz), 6.33 (1H, *s*, disappears with D_2O), 6.74 (1H, *s*), 7.68 (1H, *d*, *J* 9.5 Hz). (Found: C, 59.73; H, 4.35; M^+ 222. Calc. for $C_{11}H_{10}O_5$: C, 59.46; H, 4.54%; M^+ 222). Acetylation of **2** with Ac_2O–Py yielded from EtOH colourless prisms of 6,8-dimethoxy-7-acetoxycoumarin **5**, mp 142–143°. (Found: C, 59.38; H, 4.54; $C_{13}H_{12}O_6$ requires: C, 59.09; H, 4.58%). Methylation of **2** with CH_2N_2 yielded from EtOH faintly yellow needles of 6,7,8-trimethoxycoumarin (**3**), mp 104–105° (lit. [9] 104–105°). (Found: C, 61.48; H, 5.08. Calc. for $C_{12}H_{12}O_5$: C, 61.02; H, 5.12%). Ethylation of (**2**) with C_2H_5I–K_2CO_3 in Me_2CO gave from aq. EtOH colourless platelets of 6,8-dimethoxy-7-ethoxycoumarin **4**, mp 82.5–83.5° (lit. [8] 82°). (Found: C, 62.42; H, 5.74. Calc. for $C_{13}H_{14}O_5$: C, 62.39; H, 5.64%).

Prenylation of (**2**). Isofraxidin (**2**) (30 mg) was magnetically stirred at 80° with anhyd. K_2CO_3 (50 mg) and prenyl bromide (30 mg) in dry Me_2CO (4 ml) for 6 hr. Concentration, extraction with hot C_6H_6 and crystallization from C_6H_6–petrol (40–60°) afforded colourless platelets (11 mg), mp 89–90°. The mp was undepressed when admixed with natural product (**1**). On TLC the synthesized product had the same R_f as **1** and also gave an identical blue fluorescence under UV light (366 nm). The IR spectra of the prenylated product and (**1**) were superimposable.

Acknowledgements—We are indebted to the University and the Council for Scientific and Industrial Research for financial support. Mrs. E. Brink is thanked for assisting in the authentication of the plant material.

REFERENCES

1. Moran, V. C., Persicaner, P. H. R. and Rivett, D. E. A. (1975) *J. S. Afr. Chem. Inst.* **28**, 47.
2. Farnsworth, N. R., Pilewski, N. A. and Draus, F. J. (1962) *Lloydia* **25**, 312.
3. Steck, W. and Mazurek, M. (1972) *Lloydia* **35**, 418.
4. Bohlmann, F. and Zdero, C. (1975) *Chem. Ber.* **108**, 1902.
5. Drewes, S. E. (1974) *Chroman and Related Compounds* in *Progress in Mass Spectrometry* Vol. 2, p. 25, Verlag Chemie, Weinheim.
6. Noguti, T. and Kawanami, M. (1940) *J. Pharm. Soc. Japan* **60**, 57.
7. Ahluwalia, V. K., Gupta, V. N. and Seshadri, T. R. (1959) *Tetrahedron* **5**, 90.
8. Wessely, F. and Demmer, E. (1929) *Chem. Ber.* **62B**, 120.
9. Späth, E. and Jerzmanowska-Sienkiewiczowa, Z. (1937) *Chem. Ber.* **70B**, 1019.
10. Bohlmann, F. and Zdero, C. (1975) *Chem. Ber.* **108**, 2153.
11. Bohlmann, F., Grenz, M. and Zdero, C. (1975) *Chem. Ber.* **108**, 2955.
12. Weygand, C. (1945) *Organic Preparations*, p. 16, Interscience, New York.