Table 1. Bacteriostatic activity of coumarins and phenols

Compound	Concentration in ppm	% Colonies *E. coli*	% Colonies *B. subtilis*
Umbelliferone	65	0	0
Esculetin	65	40	—
	130	—	1
	260	—	0
Daphnetin	33	60	70
	65	0	—
	130	—	0
5,7-dihydroxycoumarin	40	0	0
Esculin	45	—	0
	180	90	—
Apterin	45	0	0
Vaginidiol	65	100	—
Oroselol	130	100	100
Oroselon	saturated	100	50
Phenol	10000	100	100
	40000	10	10
Catechol	500	—	100
	1000	—	70
	2500	35	—
	10000	—	0
	20000	1	—
Resorcinol	10000	100	—
	40000	20	30

Bacterial suspensions containing about 100 and 200 cells were pipetted into sterile plastic petri dishes. 15 ml molten and cooled nutrient agar was poured into the dishes, followed by graded vol of solutions (ethanol or acetone) of the compounds to be investigated. The soln of vaginidiol in alcohol had to be filtered through a G5 filter; it contained a high number of bacteria, most probably due to its preparation by enzymic hydrolysis of apterin. Fluids were mixed by gentle shaking and the gel was allowed to settle. Final readings (number of colonies visible) were made after 48 hr of incubation at 37°. All relevant blanks were run simultaneously. In every instance, the small amount of organic solvent (~2%) present appeared to have a negligible influence (other solvents tested were not innocuous). The bacteriostatic effect is expressed as the percentage of colonies visible vs the blank. (Table 1) Except for catechol, concentrations showing intermediate activities are not presented.

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APTERIN, A COMMON FURANOCOUMARIN GLYCOSIDE IN THE UMBELLIFERAE

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Key Word Index—Umbelliferae: apterin; 8-(2-glucosyloxy)isopropyl-9-hydroxy-8,9-dihydroangelicin.

Apterin (8-(glucosyloxy)isopropyl-9-hydroxy-8,9-dihydroangelicin) has been isolated from *Heracleum mantegazzianum* Somm, et Lev. [1] and from *Zizia aptera* [2] (both Umbelliferae). We have now found it in nine other species of this family. It is present in the roots of *Heracleum sphondylium* L., *H. laciniatum*, *H. sosnosvskyi*, *Pastinaca sativa* L., *Anthriscus silvestris* Pers. (L.) Hoffm. and *Peucedanum palustre* (L.) Moench. It is possibly present in small amounts in *Aegopodium podagraria* L., *Angelica archangelica* L. and *Levisticum officinale* (Hill) Koch, but probably not in *Myrrhis odorata* (L.) Scop., *Petroselinum crispum* (Miller) Hill and *Pimpinella saxifraga* L. However, the amount of apterin present in the roots of *H. mantegazzianum* seems to depend upon the stage of development and the growing conditions of the plant [3]. Such variations may also occur in other species, so we cannot conclude that it is necessarily absent from the three species mentioned above. The aglucone, vaginidiol, has not as yet been found in any of the plants at our disposal.

EXPERIMENTAL

Plant sources. Plants of known taxonomic authenticity were put at our disposal by the Hortus Botanicus of the University of Leyden and by the municipal "Heempark" of Leyden.

Methods. The screening method used was a small-scale version of our original isolation procedure for apterin [1]. At least three different TLC systems were used to examine the isopropanol extract for the presence of apterin; when enough apterin was present, it was purified by PLC and its identity confirmed by following (UV-spectroscopy) its acid hydrolysis (apterin → oroselol + oroselon → dimers [4]. Steck and Wetter [2] reported unsuccessful attempts to hydrolyse apterin by means of emulsin. We obtained a complete conversion with emulsin in H_2O–MeOH for 3 months. The suggestion made by Steck and Wetter about the steric hindrance protecting the glucosidic bond is supported by Röntgen-diffraction data [5].

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

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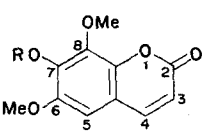
Key Word Index—*Agathosma puberula*; Rutaceae; 6,8-dimethoxy-7-prenyloxycoumarin.

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.