

sockets appeared on the bracteated costa of the wing. However, some 40% of the bristle organs located on the distal part of the femur differentiate neither a shaft nor a bract in  $H^2$ . Bracts were absent whenever a shaft was missing at positions where bracts are always formed in wild-type flies (Figure 2). On the other hand, bract were present near complete bristle organs on mutant forelegs. It is interesting that also a small bristle with a very short shaft may be accompanied by a bract of normal size (Figure 3, arrow).

Compared with  $H^2$ , the mutation  $sv^{de}$  is characterized by a higher 'cellular penetrance' in the sense that more cells exhibit a mutant phenotype. Sockets without bristles are found on adult integumental derivatives of all imaginal disks. The frequency of this extreme cellular phenotype reaches almost 100% on the thorax leading to the 'shaven' appearance of the adult fly. Where shafts do occur in the  $sv^{de}$  mutant, they rarely exhibit normal shape: in most instances they are either bent, twisted, or forked. Up to 97% of the bristle organs of the wing costa or distal leg parts did not differentiate a normal shaft. In all these cases, bracts were also missing, with a very few exceptions (< 0.1%) where sockets that lacked a shaft were nevertheless accompanied by a bract. However, most, if not all, of these rare examples must be ascribed to shafts having been lost during preparation of the flies for microscopic examination<sup>10</sup>. As in  $H^2$ , complete bristle organs were accompanied by bracts. On the other hand, bracts were absent near bristle organs that had only developed a very short shaft (Figure 4).

It has been argued<sup>11</sup> that the formation of bristles without sockets and bracts after treatment with mitomycin C or nitrogen mustard may be due to the 2 drugs inhibiting both the presumptive socket and the bract cell simultaneously and independently. Such a hypothesis is now rendered very unlikely by our observation of a perfect correlation between a normal bristle organ and the formation of an accompanying bract in the two mutants,  $H^2$  and  $sv^{de}$ . In the light of the additional evidence now available, this hypothesis would have to make the following two assumptions: 1. that 2 drugs (mitomycin C and nitrogen mustard), and 2 mutations ( $H^2$  and  $sv^{de}$ ) exert 2 independent effects on 2 different cells (tormogen/trichogen and bract cell); 2. and that the presumptive bract cell exhibits the same sensitivity as the tormogen cell towards mitomycin C and nitrogen mustard, whereas in  $H^2$  and  $sv^{de}$  it responds in the same way as the trichogen cell. We think it is much more reasonable to assume that the 2 drugs and the 2 mutations exert only one effect,

namely to inhibit the development of either the tormogen or the trichogen cell, respectively, whereas they leave unaffected the competence of the presumptive bract cell to respond to the inductive stimulus of the bristle organ. This latter conclusion is based upon the fact that in mutant flies, both  $H^2$  and  $sv^{de}$ , complete bristle organs are always accompanied by bracts, provided they are located in regions where bracts are regularly formed in wild-type flies.

If the additional data obtained with dissociated and reaggregated imaginal disks are taken into consideration, we are forced to accept as a fact that bracts in *Drosophila* are differentiated under the inductive influence emanating from a complete and normal bristle organ. In  $H^2$  even a bristle with a short shaft is capable of induction, whereas in  $sv^{de}$  this is not the case. This difference does not present any difficulties for our concept since the two mutations may well affect differently the inductive capacity of rudimentary bristle organs.

**Zusammenfassung.** Die Mutanten «Hairless<sup>2</sup>» und «shaven-depilate» von *Drosophila melanogaster* führen in den Borstenorganen zu einem Ausfall des Schaftes. Bei beiden Mutanten fehlen Borstenschaft und «bract» gleichzeitig in Regionen, wo beim Wildtyp immer «bracts» ausgebildet werden. Die Korrelation zwischen fehlendem Schaft und «bract» wird im Zusammenhang mit früheren Befunden diskutiert. Es wird der Schluss gezogen, dass nur ein vollständiges Borstenorgan die Differenzierung eines «bracts» zu induzieren vermag.

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<sup>10</sup> We feel justified to make this statement because we were able to find loose shafts somewhere else in the same preparations. Furthermore, the color and the shape of those shaft-less sockets near which a bract was nevertheless produced were the same as in complete bristle organs.

<sup>11</sup> W. GEHRING, in *Results and Problems in Cell Differentiation*, (Eds. H. URSprung and R. NÖTHIGER; Springer-Verlag, Berlin 1972), vol. 5, p. 35.

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## Coumarins of *Angelica pachycarpa*

*Angelica pachycarpa* Lge. is one of the Umbelliferae endemic to NW. Spain<sup>1-3</sup> and W. Portugal (Berlenga island)<sup>3,4</sup>. To our knowledge the plant has never been studied for its constituents<sup>5,6</sup>, despite the fact that occurrence of coumarins could be assumed. We report in this paper the identification of several of them in the plant.

**Materials and methods.** Aerial parts of the plants (300 g), exclusive of flowers and fruits, were collected in November and homogenized with methanol (1.5 l) at room temperature. After a 7-day extraction with occasional stirring, the suspension was filtered and the extraction repeated for 2 consecutive 24-h periods. The filtrates were combined, concentrated in vacuo and the residue was taken up in 60% methanol. Pigments and other fat-soluble material were removed by extraction into hexane<sup>7</sup>. The residual

60% methanolic solution was concentrated in vacuo and the methanol-free aqueous residue was continuously extracted overnight with ether.

The ether solution was dried over anhydrous sodium sulphate, concentrated and chromatographed on Whatman 3mm paper with either toluene-acetic acid-water (4:1:5 by volume; upper layer, TAW) or isopropanol-ammonia-water (10:1:1 by volume, IAW) as descending solvents. The chromatograms were observed under shortwave light (254 nm) and bands corresponding to fluorescent compounds were eluted with methanol. Overlapping of substances was showed by rechromatography in different solvents. Consequently, the eluates were sublimed at 130–190° and the sublimates were chromatographed in TAW. Several bands were eluted and

Rf values ( $\times 100$ ) and UV-data of coumarins from *A. pachycarpa*

Compound	Solvents PC									
	TAW	IAW	BBPW	H <sub>2</sub> O	HOAc	TLC MC	BW	EaC	max nm MeOH	
Unknown	35	19,28	39						269-71, 289, 313	
Ferulic acid	35	20,28	40						- 291, 314	
Unknown + marker	36	19,28	40							
Unknown	33	77	83	49	73	31	70	05	240-2, 248, 268, 311	
Byakangelicin	33	77	83	49	74	31	67	05	241, 248, 268, 312-3	
Unknown + marker	33	76	82	49	73	33	68	05		
Unknown	10	53	87	41	60	36	86	55	249, 330	
Umbelliferone	10	53	87	43	60	37	90	56	245 <sup>s</sup> , 325	
Unknown + marker	—	—	87	—	60	35	87	—		
Unknown	56	87	84	55	75	56	84	14	255, 330	
Marmesin	54	87	86	62	74	53	84	15	258 <sup>s</sup> , 335	
Unknown + marker	—	—	—	—	—	56	84	15		
Unknown	16	80	80	43	72	32	74	03	-, 249-50, -, 311	
Oxypeucedanin hydrate	16	80	83	45	73	32	74	03	222, 250, 268, 310	
Unknown + marker	15	80	82	45	72	32	74	03		
Unknown	79	89	88	40	69	65	40			
Ostruthol	83	90	90	43	67	66	38			
Unknown + marker	82	89	—	40	68	68	—			

BBPW, butanol-benzene-pyridine-water (10:2:6:3); HOAc, 10% acetic acid; MC, methanol-chloroform (5:95); BW, butanol saturated with water; EaC, ethyl acetate-ciclohexane (35:65); S, shoulder.

eluates were either allowed to crystallize overnight or chromatographed.

The compounds were characterized by UV-spectroscopy, co-chromatography (Table) and chemical reactions with Emerson reagent or sulfanilic acid<sup>8</sup>. When crystals were obtained, identifications were confirmed by m.p. and m.s.

**Results and discussion.** *A. pachycarpa* proved to be a plant extremely rich in coumarins since more than 12 were detected in it. Byakangelicin (1.3 mg/100 g; m.p. 115-7°. Parent mass 334) and oxypeucedanin hydrate (0.7 mg/100 g; m.p. 133°. Parent mass 304 and fragmentation pattern identical to that of an authentic sample) were present in substantial amounts. Umbelliferone, marmesin and ostruthol were identified, as well as ferulic acid.

This array of compounds is quite attractive from a biosynthetic standpoint, for their co-occurrence is quite consistent with the hypothetical pathway proposed for the biosynthesis of linear furanocoumarins<sup>9</sup>. Special care was taken that psoralen, a crucial intermediate in the biogenesis, was not overlooked, but it was not detected. On the other hand, this furanocoumarin is rather rare in *Angelica* spp. (4 out of 28 species)<sup>6</sup>.

From a chemotaxonomic point of view, all coumarins occurring in *A. pachycarpa* were previously found in *Angelica* spp.<sup>6</sup> and no geographical differences seem to take place<sup>10</sup>.

**Resumen.** Acido ferúlico, byakangelicina, umbeliferona, marmesina, hidrato de oxypeucedanina y ostruthol se identificaron en *A. pachycarpa*, umbelífera endémica de

España y Portugal. Se hacen consideraciones biosintéticas sobre su presencia conjunta.

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