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TEN AZULENES FROM *PLAGIOCHILA LONGISPINA* AND *CALYPOGEIA AZUREA**

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Key Word Index—*Plagiochila longispina*; *Calypogeia azurea*; Jungermanniales; Hepaticae; azulenes; synthesis.

Abstract—From gametophytic tissues of the naturally grown foliose liverworts *Plagiochila longispina* and *Calypogeia azurea* and from *in vitro* cultures of *C. azurea*, 10 azulenes have been isolated, of which eight proved to be new natural products. Their structures were elucidated by means of spectroscopic methods and by independent synthesis.

INTRODUCTION

Azulenes are known from tracheophytes, mainly angiosperms, where they often occur together with terpenoids, being synthesized in the plant by dehydrogenation of sesquiterpenes [1]. Liverworts are the only bryophyte class from which azulenes have been reported: 1,4-dimethylazulene and 1-methoxycarbonyl-4-methylazulene are the only known such constituents so far detected in liverwort species. In all liverworts, where it occurs, 1,4-dimethylazulene is either the main or the only azulene. Huneck [2] reported, for the first time, the occurrence of azulenes in the blue oil bodies of liverworts: he isolated both compounds from *Calypogeia azurea*. Takeda and Katoh [3] also detected 1,4-dimethylazulene in extracts of cell suspension cultures of *C. granulata*. Katoh and Takeda [4] and Nagashima *et al.* [5] found the same azulene in *C. peruviana*, *C. tosana*, *Macrolejeunea pallescens* and *Plagiochila micropterys*.

In the course of a chemical study of the liverwort *Plagiochila longispina* [6], we noticed the occurrence of a blue azulene in its methylene chloride extract. A closer investigation of this extract revealed, besides the two main azulenes, the occurrence of further minor azulenes. A reinvestigation of *Calypogeia azurea* from the natural habitat and from *in vitro* cultures surprisingly revealed even more azulenes as minor constituents in the methylene chloride and MeOH extracts from both sources.

RESULTS AND DISCUSSION

Azulenes from Plagiochila longispina

A *n*-hexane–methylene chloride extract (see Experimental) of *Plagiochila longispina* revealed, after chromatographic separation, the presence of one blue and three purple compounds. The blue compound was isolated by column chromatography on silica gel with *n*-hexane, whereas this solvent did not elute the three further ones from the column. The mass spectrum of **1** showed *m/z* 156 as $[M]^+$ and the ^1H NMR spectrum revealed the same signal pattern and chemical shifts as published by Meuche and Huneck [7] for 1,4-dimethylazulene. This compound (*ca* 5 mg) was the main azulene type of the lipophilic extract; the three further ones could be isolated only in traces. The real content of **1** in the liverwort is certainly much higher, because it is very volatile and most of it evaporated from the air-dried plant material after its collection in the Ecuadorian Andes. The purple colour of the three further constituents indicated the presence of an oxygen substituted azulene skeleton [1]. Compound **2** was isolated from the silica gel column by a *n*-hexane–ethyl acetate gradient, eluted with 5% ethyl acetate together with the chlorophylls and was separated from them by column chromatography on RP 18 with 80% aqueous methanol.

Compounds **3** and **4** eluted from the silica gel column with more than 5% ethyl acetate, **4** only after addition of 5% acetic acid to the solvent. Both compounds were finally separated by column chromatography on silica gel with *n*-hexane–10% ethyl acetate as solvent (see Experimental).

The spectroscopic data of **2** are in accord with those published by Meuche and Huneck [7] for 1-methoxycarbonyl-4-methylazulene and identical in all respects with an authentic sample synthesized according to the literature procedure [8] from 4-methylazulene (**5**). For the other two compounds, the structures of 1-formyl-4-methyl-azulene (**3**) and 1-carboxy-4-methoxymethyl-azulene (**4**) can be assigned mainly on the basis of mass and ^1H NMR spectroscopic data (Table 1). An unambiguous structure proof for **3**/**4** is provided by the independent synthesis of **3** from 4-methylazulene **5** and of **4** from ester **2**. These are new natural products.

After this first isolation of oxygen-containing azulenes from *Plagiochila longispina*, we reinvestigated *Calypogeia azurea* to see if it contained any new azulenes [2]. We analysed both gametophytic fresh material of *C. azurea*

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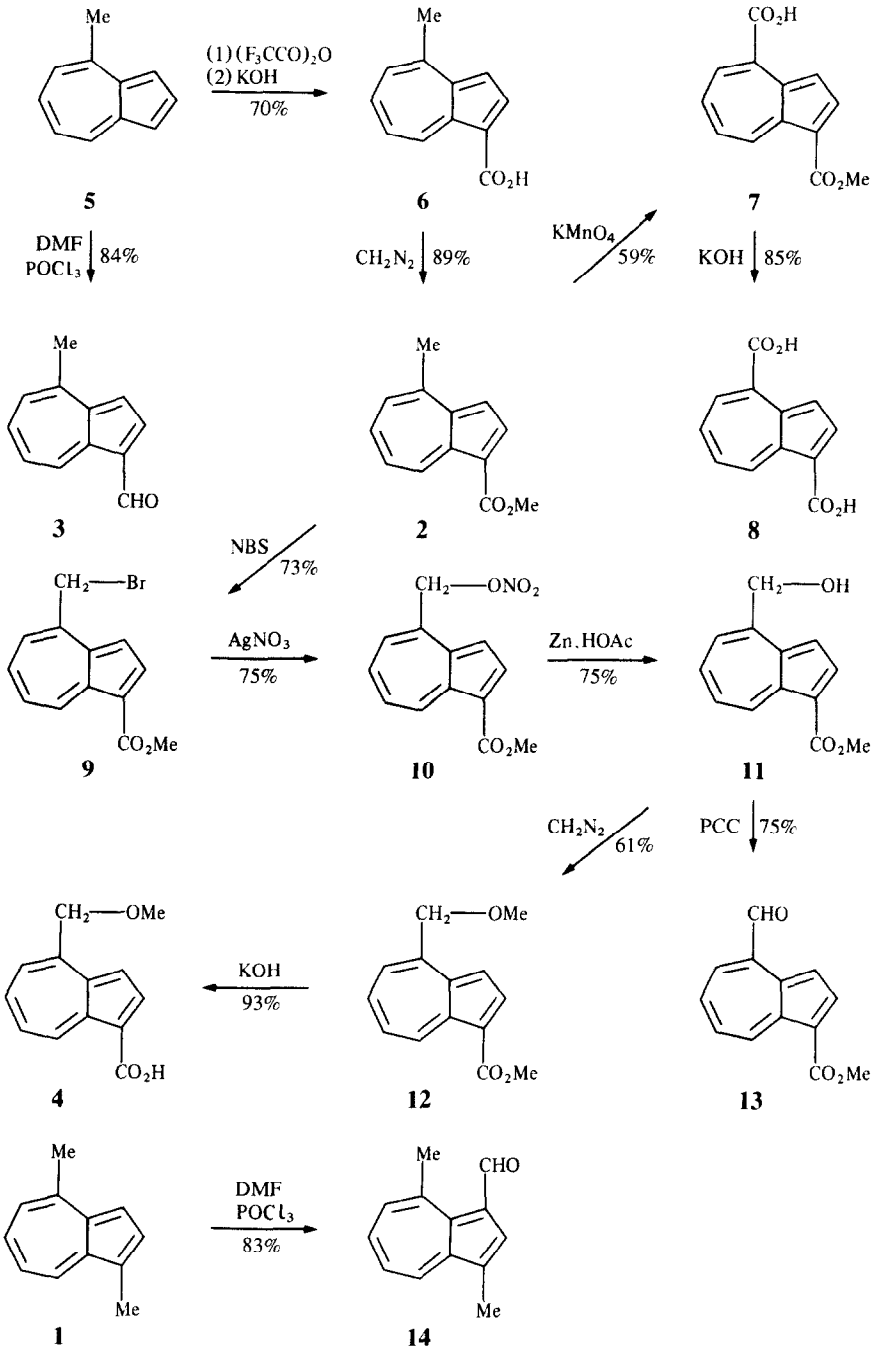

collected from the natural habitat (see Experimental) and air-dried material from *in vitro* cultures.

Azulenes from Calypogeia azurea

As the *in vitro* cultures generally contained more compounds than the naturally grown plants, we analysed the *in vitro* cultures. The lipophilic extracts were separated with a column chromatography system (see Experimental) by which seven compounds were eluted almost in the front, followed by two others. The ^{1}H NMR, mass spectra and chromatographic data (Table 1) proved the blue compound to be the 1,4-dimethylazulene (**1**). With 90 mg (=0.05% of dry weight of extracted plant material) this was the main azulene of *C. azurea* from *in vitro* cultures. The second main constituent was shown to be 1-methoxycarbonyl-4-methylazulene (**2**) by its spectroscopic data (Table 1). The yield was 28 mg (=0.02% of dry weight of extracted plant material).

The first seven compounds were separated by column chromatography with a gradient system, in which three azulenes eluted first, followed by four others (see Experi-

mental). The yield of these and the following pure compounds was 1 to 2 mg or less. Compounds **8** ester and **11** are a red purple and **7** a deep purple colour. In solid state they are relatively sensitive to oxidation, thus they are better stored in solution, e.g. in acetone. These compounds are 1,4-disubstituted azulenes according to their spectroscopic data (Table 1): one proved to be 4-hydroxymethyl-1-carboxymethyl-azulene (**11**) and another 4-carboxy-1-methoxycarbonylazulene (**7**), as shown by their independent synthesis. The ester of **8** was rather unstable being easily hydrolysed to the stable azulene-1,4-dicarboxylic acid (**8**), obtained independently by saponification of the monoester **7**. We assume that in the natural product the carboxy group at position 4 is esterified with a hitherto unknown alcohol, since ^{1}H NMR spectroscopic data indicate a free carboxy group at position 1.

The purification of the second fraction with four compounds resulted in the isolation of azulenes **3** and **6** as main compounds and of **13** and **14** as trace constituents. The ^{1}H NMR, mass spectra and chromatographic data of **3** are identical with the same compound isolated from *Plagiochila longispina* thus identifying it as 4-methylazulene-1-aldehyde. As **8** ester and **7**, **6** shows the typical chromatographic behaviour of an acid. By direct comparison of its spectroscopic data with an authentic sample synthesized according to ref. [8] it was shown to be 4-methylazulene-1-carboxylic acid.

Although **13** and **14** were only isolated in minute amounts, the structure of **14** could be completely elucidated, whereas for **13** full spectroscopic characterization was not possible. Compound **14** appeared chromatographically as a carboxy-free blue-purple azulene. Among all azulenes discussed in this paper the ^{1}H NMR spectrum of **14** exhibits for the first time a structure type different from 1,4-disubstitution: the ^{1}H NMR and mass spectral data show two methyl groups and an aldehyde function arranged in the pattern of a 1,3,4-trisubstituted azulene skeleton. The independent synthesis by formylation of 1,4-dimethylazulene (**1**) proves its structure to be 1,4-dimethyl-3-formyl-azulene.

Compound **13** could not be purified enough to get suitable ^{1}H NMR and mass spectra because of the minute amount available. It contained, as a chromatographically detectable impurity, 4-methyl-azulene-1-carboxylic acid (**6**). For **13** the structure of a 4-formyl-1-methoxycarbonylazulene seems to be likely, since its fragmentation pattern in the mass spectrum (Table 1) is identical with that of the product **13** synthesized by oxidation of the hydroxymethyl compound **11**. Further information on **13** is not available. A formyl methoxycarbonylazulene was found in *Helichrysum* and *Ixiolaena* species (Compositae) [9–11], the physical and spectroscopic data of which are not in accord with our findings on **13**. Compounds **6**, **7**, **8** ester, **11**–**14** are new natural products.

From the material of the natural habitat we isolated the azulenes **1**–**3** and **14**. With 2300 mg (=0.45% of extracted air-dried plant material) the yield of the main compound **1** was significantly higher than for the cultures. The second main compound **2** was obtained in an amount of 52 mg (=0.01%), almost the same yield as for the cultured material, and the two remaining compounds were isolated only in traces. Thus, besides compound **1**, which was found in almost 10 times higher yield in the naturally grown plants than in those from *in vitro* cultures, the quantity of the isolated azulenes is comparable in both sources. From *in vitro* cultures, nine azulenes have been isolated, whereas only four azulenes, structurally identical to those from *in vitro* cultures, were detected in the plants collected in the field.

The independent syntheses were performed as outlined in the Experimental. Thus, aldehyde **3** was obtained by Vilsmeier formylation of 4-methylazulene (**5**) with DMF–$POCl_3$, the monoester **7** by oxidation of known **2** [8] with $KMnO_4$ and the diacid **8** by saponification of **7** with KOH in methanol–H_2O. The ester **2** also served as starting material for the synthesis of **4** by functionalization at the 4-methyl group through NBS-bromination (**2**→**9**), nucleophilic exchange of bromine by nitrate (**9**→**10**) and reductive cleavage of the nitrate **10** by Zn in acetic acid to give the 4-hydroxymethyl ester **11**. The ester **11** was successively *O*-methylated with diazomethane (to yield **12**) and saponified with KOH in methanol –H_2O to give rise to the acid **4** with an overall yield of 22% (based on **2**, in a five-step sequence). Oxidation of the hydroxymethyl ester **11** by PCC leads to the aldehyde ester **13**. Finally, formylation of **1** (DMF–$POCl_3$) gave rise to the aldehyde **14**.

EXPERIMENTAL

Plant material. Fresh gametophytes of *Plagiochila longispina* Lindenb. et Gott. were collected in October 1988 in the Ecuadorian Andes, Prov. Napo, road Quito-Baeza, subalpine disturbed rainforest, 3400 m, by J.-P. Frahm and S. R. Gradstein (No. 6929). Gametophytic material of *Calypogeia azurea* Stotler et Crotz was collected from the natural habitat in August 1990 in the Bernese Alps, near Handegg, Hasli-valley, Switzerland, at about 1500 m by U.S. and R.M. Voucher specimens are deposited in the herbarium of the Fachrichtung Botanik, Universität des Saarlandes, Saarbrücken (SAAR). The plants were identified by S. R. Gradstein (Utrecht; P.l.) and R.M.(C.a.). An axenic culture of *C. azurea* was obtained in 1985 from Dr. Vandekerkhove, University of Mainz. The cultures were grown in 200-ml flasks with 50 ml solid modified B5 (pH 5.7) medium [12], containing 20 $g\,l^{-1}$ sucrose. The flasks were kept under constant illumination (2000 lux) at 20°.

Extraction and isolation.

Plagiochila longispina. After careful cleaning, air-dried gametophytes (30 g) were ground in a coffee-mill and extracted with CH_2Cl_2. This extract was evapd to a small vol. and chromatographed by CC over silica gel with a *n*-hexane–Me_2CO gradient. Compound **1** was eluted by *n*-hexane and purified again by a silica gel column with *n*-hexane. Compounds **2**–**4** eluted from the silica gel column with a gradient of *n*-hexane–EtOAc starting from *n*-hexane up to *n*-hexane–EtOAc (17:3). Chlorophyll and other lipids were separated from the azulenes by CC on RP 18 with 80% aq. MeOH, in which the azulenes eluted, and compound **2** was separated from **3** and **4**. Final purification of **2** was achieved by CC on silica gel with *n*-hexane–EtOAc (9:1). The yield was less than 1 mg.

Compounds **3** and **4** were separated from each other on a silica gel column with *n*-hexane–EtOAc (9:1). With this solvent **3** eluted and was finally purified by prep. HPLC on a Spherisorb ODS-2 column with MeCN–H_2O (1:1; R_t 20 min) in about 1 mg amount. Addition of 5% HOAc to the last silica gel column eluted **4** in traces. It was finally purified two times by CC on Sephadex LH-20 with MeOH (Uvasol)–H_2O (bidest.; 2:3).

Calypogeia azurea, in-vitro *cultured material.* Air-dried gametophytes (160 g) were ground in a coffee-mill and extracted first with *n*-hexane, followed by CH_2Cl_2 and EtOAc. The combined extracts were evapd to a small vol. and chromatographed by CC

Table 1. Spectroscopic data of azulenes **1–4/6–14***

Compound	IR ν_{max}^{KBr} cm^{-1}	UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε)	^{1}H NMR (400 MHz, $CDCl_3$)	^{13}C NMR (100 MHz, $CDCl_3$)	CIMS (probe) 120 eV, *m/z* (rel. int.)
1	3020, 2920 1595, 1460	722 (2.90) 625 (3.33) 597 (3.42) 362 (4.31) 347 (4.63) 337 (4.80)	δ8.21 (1H, *d*, *J* = 9.5 Hz, H-8) 7.64 (1H, *d*, *J* = 4.7 Hz, H-2) 7.43 (1H, *t*, *J* = 9.9 Hz, H-6) 7.31 (1H, *d*, *J* = 3.7 Hz, H-3) 7.05–6.97 (2H, *m*, H-5/H-7) 2.84, 2.65 (3H, *s*, Me)	δ145.4, 141.2, 139.9, 138.6, 137.4, 126.8, 126.2, 126.0, 124.8, 116.3, 24.3, 19.8	157 ([M + 1]$^+$, 89) 156 ([M]$^+$, 100) 141 (18) 115 (4)
2	2945, 1690 (CO), 1460, 1145	602 (3.33) 553 (3.58) 348 (4.68) 332 (4.55)	δ9.69 (1H, *d*, *J* = 9.8 Hz, H-8) 8.31 (1H, *d*, *J* = 4.3 Hz, H-2) 7.69 (1H, *t*, *J* = 9.9 Hz, H-6) 7.51–7.39 (2H, *m*, H-5/H-7) 7.30 (1H, *d*, *J* = 4.3 Hz, H-3) 3.94 (3H, *s*, CO_2Me) 2.94 (3H, *s*, Me)	δ166.0 (CO), 148.5, 142.6, 140.4, 139.0, 137.9, 132.7, 130.1, 126.4, 117.2, 115.1, 51.0 (CO_2Me), 25.0 (Me)	201 ([M + 1]$^+$, 11) 200 ([M]$^+$, 21) 185 (7) 142 (27) 115 (100)
3	2945, 2725, 1650 (CO), 1265	630 (2.42) 533 (2.90) 384 (4.18) 370 (4.17) 305 (4.87)	δ10.35 (1H, *s*, CHO) 9.64 (1H, *d*, *J* = 9.6 Hz, H-8) 8.21 (1H, *d*, *J* = 4.2 Hz, H-2) 7.76 (1H, *t*, *J* = 10.1 Hz, H-6) 7.60–7.50 (2H, *m*, H-5/H-7) 7.36 (1H, *d*, *J* = 4.2 Hz, H-3) 2.97 (3H, *s*, Me)	δ186.8 (CO), 149.9, 144.3, 141.3, 139.8, 138.6, 137.8, 131.5, 128.3, 126.4, 116.7, 25.1 (Me)	171 ([M + 1]$^+$, 10) 170 ([M]$^+$, 64) 169 (100) 115 (58)
4	3415 (OH), 2920, 1710 (CO), 1030	640 (2.15) 402 (3.67) 385 (4.36) 321 (4.68) 301 (4.54)	δ9.69 (1H, *d*, *J* = 9.6 Hz, H-8) 8.30 (1H, *d*, *J* = 4.3 Hz, H-2) 7.86 (1H, *t*, *J* = 9.6 Hz, H-6) 7.63–7.49 (2H, *m*, H-5/H-7) 7.24 (1H, *d*, *J* = 4.3 Hz, H-3) 3.61 (3H, *s*, OMe) 3.59 (2H, *s*, CH_2)	δ165.3 (CO), 148.7, 142.3, 140.8, 140.0, 139.7, 138.1, 133.9, 130.4, 126.3, 117.6, 69.3, 52.5	217 ([M + 1]$^+$, 5) 216 [M]$^+$, 8) 172 (100) 126 (21) 115 (34)
6	3445 (OH), 2915, 1640 (CO), 1250	548 (3.40) 347 (4.51) 330 (4.36) 300 (4.93)	δ9.64 (1H, *d*, *J* = 9.8 Hz, H-8) 8.24 (1H *d*, *J* = 4.2 Hz, H-2) 7.86 (1H, *t*, *J* = 9.6 Hz, H-6) 7.62–7.54 (2H, *m*, H-5/H-7) 7.39 (1H, *d*, *J* = 4.2 Hz, H-3) 2.94 (3H, *s*, Me)†	δ166.2 (CO), 148.9, 141.8, 139.4, 138.8, 138.3, 137.3, 130.1, 126.1, 117.2, 114 9, 24.5 (Me)†	187 ([M + 1]$^+$, 11) 186 ([M]$^+$, 100) 169 (81) 144 (38) 115 (91)
7	3310 (OH), 2915, 1720/1695 (CO),	621 (2.98) 399 (3.88) 315 (4.61)	δ9.80 (1H, *d*, *J* = 9.9 Hz, H-8) 8.21 (1H, *d*, *J* = 4.2 Hz, H-2) 7.86 (1H, *t*, *J* = 9.9 Hz, H-6)	δ168.4 (CO), 165.9 (CO), 148.9, 141.5,	230 ([M]$^+$, 26) 199 (45) 186 (100)‡

	1215, 1040	309 (4.47) 301 (4.46)	7.73–7.59 (2H, *m*, H-5/H-7) 7.41 (1H, *d*, J = 4.2 Hz, H-3) 3.95 (3H, *s*, CO_2Me)	140.1, 138.8, 138.2, 137.2, 131.5, 130.6, 127.9, 117.4, 51.0 (Me)	
8	3390, 2980, 1750/1695 (CO), 1460, 1035	633 (2.71) 501 (2.99) 399 (4.01) 336 (4.48) 300 (4.85)	δ9.86 (1H, *d*, J = 9.8 Hz, H-8) 8.81 (1H, *s*, CO_2H) 8.35 (1H, *d*, J = 4.4 Hz, H-2) 7.84 (1H, *t*, J = 9.7 Hz, H-6) 7.61–7.49 (2H, *m*, H-5/H-7) 7.40 (1H, *d*, J = 4.4 Hz, H-3)	δ168.5 (CO), 166.3 (CO), 141.6, 140.5, 139.8, 138.8, 137.1, 136.9, 129.6, 128.3, 124.4, 117.2	216 ([M]$^+$, 16) 198 (7) 172 (11) 115 (100)‡
9	2940, 1695 (CO), 1440, 1095	662 (3.82) 361 (4.51) 349 (4.76) 314 (4.96)	δ9.76 (1H, *d*, J = 9.3 Hz, H-8) 8.38 (1H, *d*, J = 4.4 Hz, H-2) 6.76 (1H, *t*, J = 10.0 Hz, H-6) 7.54–7.46 (2H, *m*, H-5/H-7) 7.26 (1H, *d*, J = 4.4 Hz, H-3) 4.47 (2H, *s*, CH_2) 3.94 (3H, CO_2Me)	δ164.5 (CO), 147.5, 145.4, 142.2, 139.5, 138.4, 134.5, 132.3, 128.1, 119.4, 117.4, 51.4 (Me), 34.0 (CH_2)	
10	2930, 1690 (CO), 1635/1450 (NO_2), 1265, 1075	656 (3.79) 363 (4.46) 346 (4.72) 310 (4.90)	δ9.71 (1H, *d*, J = 9.4 Hz, H-8) 8.32 (1H, *d*, J = 4.4 Hz, H-2) 7.71 (1H, *t*, J = 9.9 Hz, H-6) 7.60–7.49 (2H, *m*, H-5/H-7) 7.30 (1H, *d*, J = 4.4 Hz, H-3) 4.62 (2H, *s*, CH_2) 3.94 (3H, *s*, CO_2Me)	δ165.0 (CO), 148.4, 145.6, 143.0, 140.2, 139.5, 135.7, 132.4, 129.0, 121.0, 117.7, 68.3 (CH_2), 51.0 (Me)	
11	3310 (OH), 2915, 1695 (CO), 1465, 1030	657 (2.31) 355 (3.81) 343 (4.31) 318 (4.22)	δ9.79 (1H, *d*, J = 9.7 Hz, H-8) 8.36 (1H, *d*, J = 4.4 Hz, H-2) 7.74 (1H, *t*, J = 9.9 Hz, H-6) 7.71–7.59 (2H, *m*, H-5/H-7) 7.31 (1H, *d*, J = 4.4 Hz, H-3) 3.92 (3H, *s*, CO_2Me) 3.75 (2H, *s*, CH_2) 3.90 (1H, *s*, OH)	δ166.5 (CO), 148.6, 143.0, 141.0, 140.9, 139.3, 137.8, 132.7, 130.9, 126.4, 117.5, 62.2 (CH_2), 51.0 (Me)	217 ([M+1]$^+$, 8) 216 ([M]$^+$, 21) 198 (100)
12	2930, 1690 (CO), 1250, 1035	675 (2.81) 411 (4.07) 382 (3.99) 305 (4.68)	δ9.70 (1H, *d*, J = 9.8 Hz, H-8) 8.41 (1H, *d*, J = 4.4 Hz, H-2) 7.77 (1H, *t*, J = 9.8 Hz, H-6) 7.69–7.55 (2H, *m*, H-5/H-7) 7.29 (1H, *d*, J = 4.4 Hz, H-3) 3.93 (3H, *s*, CO_2Me)	δ167.3 (CO), 149.1, 142.9, 141.7, 141.0, 139.6, 137.3, 133.0, 130.9, 126.7, 117.4,	231 ([M+1]$^+$, 11) 230 ([M]$^+$, 16) 186 (100) 128 (27)

Table 1. *Continued*

Compound	IR ν_{max}^{KBr} cm^{-1}	UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε)	^{1}H NMR (400 MHz, $CDCl_3$)	^{13}C NMR (100 MHz, $CDCl_3$)	CIMS (probe) 120 eV, *m/z* (rel. int.)
			3.79 (3H, *s*, OMe) 3.51 (2H, *s*, CH_2)	69.3 (CH_2), 51.2, 50.9 (Me)	
13	2930, 2720, 1700/1690 (CO), 1450, 1030	599 (2.72) 394 (4.16) 331 (4.53) 309 (4.61)	δ10.31 (1H, *s*, CHO) 9.66 (1H, *d*, *J*=9.2 Hz, H-8) 8.19 (1H, *d*, *J*=4.6 Hz, H-2) 7.75 (1H, *t*, *J*=9.2 Hz, H-6) 7.66–7.51 (2H, *m*, H-5/H-7) 7.31 (1H, *d*, *J*=4.6 Hz, H-3) 3.95 (3H, *s*, CO_2Me)	δ205.4 (CO), 166.4 (CO), 148.2, 141.3, 140.8, 139.9, 139.6, 138.0, 133.2, 130.6, 125.4, 118.1, 52.4 (Me)	214 ([M]$^+$, 21) 186 (43) 185 (27) 170 (51) 159 (31) 157 (20) 156 (14) 127 (29) 115 (100)‡
14	2950, 2725, 1645 (CO), 1465, 1220, 1050	675 (2.71) 506 (3.42) 384 (4.45) 320 (4.83) 312 (4.83) 306 (4.80)	δ10.66 (1H, *s*, CHO) 8.32 (1H, *d*, *J*=9.4 Hz, H-8) 8.23 (1H, *s*, H-2) 7.62 (1H, *t*, *J*=9.6 Hz, H-6) 7.45–7.33 (2H, *m*, H-5/H-7) 3.15, 2.65 (3H, *s*, Me)	δ195. 4 (CO), 148.6, 146.7, 139.4, 137.3, 136.5, 136.2, 135.9, 127.0, 125.9, 119.4, 24.8 (Me), 16.4 (Me)	185 ([M]$^+$ +1, 7) 184 ([M]$^+$, 67) 156 (17) 153 (21) 115 (100)

*Full spectroscopic characterization is also given for the known compounds **1**, **2** and **6**.

†Measured in DMSO as solvent.

‡EIMS (probe), 70 eV, *m/z* (rel. int.).

over RP 18 with 90% aq. MeOH. The minor azulenes eluted in the front, followed by the first main compound **2** and the second main compound **1**, which eluted just before the chlorophyll and the lipids. Compound **1** was removed from the eluent by solid-phase extraction on RP 18, eluted with Me_2CO and finally purified on a silica gel column with *n*-hexane. Final purification of **2** was achieved by CC on silica gel with *n*-hexane–EtOAc (9:1). The other compounds were separated on a RP 18 column (Lobar B, Merck, Darmstadt) with a gradient of 50% aq. MeOH to pure MeOH in presence of 5% HOAc. Among the 10 collected fractions, 2 and 3 contained the azulenes **7**, **8** ester and **11**, 4 contained **3**, **6**, **13** and **14**. The first compounds were separated by CC on silica gel with *n*-hexane–EtOAc (3:2). With this solvent **11** eluted; after addition of 5% aq. HOAc first **8** ester and later **7** eluted too. Final purification was performed in each case by CC on silica gel with *n*-hexane–EtOAc–HOAc (16:3:1) followed by CC on Sephadex LH-20 with MeCN–5% aq. HOAc (3:2). After evapn to dryness only about half of **8** ester was still soluble in Me_2CO. The other half could only be dissolved in MeOH or H_2O. This behaviour indicated a chemical change to a more polar compound, which was later found to be identical with the dicarboxylic acid **8**.

Compound **7** also decomposed partly on the Sephadex LH-20 column to a polar product, later proved to be identical with **8**. Fraction 4 with four compounds was shaken with *n*-hexane to separate the azulenes from any more polar constituents. After evapn to a smaller vol. the *n*-hexane phase was separated on a silica gel column with *n*-hexane–EtOAc–HOAc (17:2:1) resulting in the pure compounds **6** and **13**, **3** and **14** still being in a mixture. Both compounds were finally separated on a silica gel column with *n*-hexane–EtOAc (17:3).

Calypogeia azurea *from the natural habitat*. After cleaning, fresh gametophytes (5 kg) were ground and extracted with Me_2CO. The extract was evapd to the H_2O phase and shaken against EtOAc until the H_2O phase was free of azulenes. The EtOAc phase was evapd to a small vol. and chromatographed by CC over silica gel with a gradient from *n*-hexane up to a mixture of *n*-hexane–EtOAc (4:1). Compound **1** eluted with *n*-hexane and was finally purified by two silica gel columns with *n*-hexane (2300 mg). The oxygen-containing azulenes **2**, **3** and **14** were separated from chlorophyll and the lipids by CC on RP 18 with MeOH–H_2O–HOAc (18:1:1). The azulene fraction was subsequently chromatographed on a silica gel column with a gradient of *n*-hexane up to *n*-hexane–EtOAc (7:3). With this column **2** was separated from the last 2 compounds **3** and **14**. Final purification of **2** was achieved by CC on silica gel with *n*-hexane–EtOAc (9:1; 28 mg). Compounds **3** and **14** were separated by CC on silica gel with *n*-hexane–EtOAc (17:3), **14** eluting first. Compound **3** was finally purified by CC on RP 18 (Lobar B, Merck, Darmstadt) with MeOH–H_2O (3:2) (*ca* 2 mg). Because of the minute amount (< 1 mg), **14** had to be purified by micro-prep. HPTLC on RP 18 (Merck, Darmstadt, 10 × 10 cm) with 90% aq. MeOH.

TLC: silica gel; *n*-hexane; *n*-hexane–EtOAc (19:1); 2D-TLC on RP 18 (Merck, Darmstadt, 5 × 5 cm); 1: *n*-hexane–propionic acid (9:1); 2: MeOH–H_2O–propionic acid (14:5:1). CC: silica gel; 25 μ for MPLC (Merck, Darmstadt); RP 18; 25–40 μ Baker (for flash chromatogr.); for solvents see above. HPLC: 1. prep. column: Spherisorb ODS-2; 5 μ; 250 × 20 mm^2, flow rate 10 ml min^{-1}; UV: 280 nm; solvents see above; 2. separation of isolated azulenes was achieved on Superspher RP-Select B (Merck, Darmstadt); 5 μ; 250 × 4 mm; flow rate 1 ml min^{-1}; UV: 280 nm; solvent: linear gradient of 50% to 90% aq. MeOH in 1% phosphoric acid within 20 min, followed by 8 min isocratic flow with the last solvent.

1-*Methoxymethyl*-4-*methylazulene* (**2**). The acid **6** (0.50 g, 2.69 mmol) in MeOH (20 ml) is esterified with CH_2N_2 in Et_2O {prepared from *N*-nitrosomethyl urea (2.00 g, 19.4 mmol) according to the procedure in [13]}. Usual workup and purification by CC (SiO_2, eluent Et_2O) yields the product as violet crystals; 0.48 g **2** (89%), mp 49–51°. Found: C, 78.11; H, 6.09; O, 15.98. $C_{13}H_{12}O_2$ (200.2); requires: C, 77.98; H, 6.04; O, 16.12. The spectroscopic data (see Table 1) are in accord with the data reported in ref. [8].

1-*Formyl*-4-*methylazulene* (**3**). 4-Methylazulene **5** [14] (1.50 g, 10.5 mmol), dimethylformamide (20 ml) and $POCl_3$ (1.84 g, 12.0 mmol) were reacted according to the procedure in ref. [15]. After the usual workup and purification by CC (neutral Al_2O_3, activity grade I, 150 g; eluent Et_2O) the product was obtained as deep-red oil, 1.50 g **3** (84%). Found: C, 84.60; H, 5.99; O, 9.29. $C_{12}H_{10}O$ (170.2); requires: C, 84.68; H, 5.92; O, 9.40. The spectroscopic characterization is given in Table 1.

1-*Carboxy*-4-*methoxymethyl azulene* (**4**). The ester **12** (0.25 g, 1.09 mmol) was saponified with KOH (0.55 g, 9.80 mmol) in MeOH–H_2O (35 ml–35 ml) according to the procedure in ref. [16]. Usual workup yields the product as violet crystals, which are recrystallized from EtOH; 0.22 g **4** (93%), mp 128–129°. Found: C, 72.00; H, 5.40; O, 22.8. $C_{13}H_{12}O_3$ (216.2); requires: C, 72.21; H, 5.59; O, 22.20. The spectroscopic characterization is given in Table 1.

1-*Carboxy*-4-*methylazulene* (**6**). 1-Trifluoroacetyl-4-methylazulene [8] (1.00 g, 4.20 mmol) was reacted with KOH (2.35 g, 41.9 mmol) in EtOH–H_2O (15 ml–15 ml) according to the procedure in ref. [8]. Usual workup yields the product as violet needles, which are recrystallized from Et_2O–*n*-hexane 1:1; 0.69 g **6** (88%) mp 189–190°C. Found: C, 77.21; H, 5.30; O, 17.09. $C_{21}H_{10}O_2$ (186.2); requires: C, 77.40; H, 5.41; O, 17.18. The spectroscopic characterization is given in Table 1.

4-*Carboxy*-1-*methoxycarbonylazulene* (**7**). The ester **2** (0.35 g, 1.75 mmol) was oxidized by $KMnO_4$ (0.85 g, 5.38 mmol) in MeOH (50 ml) according to the procedure in ref. [16]. After the usual workup the product was obtained as blue-violet crystals, which are recrystallized from EtOH; 0.24 **7** (59%), mp 159–161°. Found: C, 67.70; H, 4.30. $C_{13}H_{10}O_4$ (230.2); requires: C, 67.82; H, 4.38. The spectroscopic characterization is given in Table 1.

1,4-*Dicarboxyazulene* (**8**). The monoester **7** (0.20 g, 0.87 mmol) was saponified with KOH (0.50 g, 8.91 mmol) in MeOH–H_2O (35 ml–35 ml) according to the procedure in ref. [16]. Usual workup gives the product as violet crystals, which are recrystallized from EtOH; 0.16 g **8** (85%), mp 120–122°. Found: C, 66.48; H, 3.63. $C_{12}H_8O_4$ (216.2); requires: C, 66.67; H, 3.73. The spectroscopic characterization is given in Table 1.

4-*Bromomethyl*-1-*methoxycarbonylazulene* (**9**). The ester **2** (1.00 g, 5.00 mmol), *N*-bromosuccinimide (0.92 g, 5.16 mmol) and azoisobutyronitrile (0.20 g) in CCl_4 (120 ml) were reacted according to the procedure in ref. [16]. Usual workup yields the product as black-blue platelets, which are recrystallized from petrol ether (40–60°); 1.02 g **9** (72%), mp 113–114°. Found: C, 55.79; H, 3.90. $C_{13}H_{11}BrO_2$ (279.1); requires: C, 55.94; H, 3.97. The spectroscopic characterization is given in Table 1.

1-*Methoxycarbonyl*-4-*nitromethylazulene* (**10**). A soln of the bromide **9** (0.88 g, 3.16 mmol) in MeCN (100 ml) was added slowly with stirring to the soln of $AgNO_3$ (0.56 g, 3.28 mmol) in MeCN (20 ml). After 1 hr at reflux temp. and 2 hr at 20° the precipitate (AgBr) was filtered off and the filtrate evapd to dryness. The product was obtained as black-blue needles, which are recrystallized from EtOH; 0.62 g **10** (75%), mp 138–140°. Found: C, 59.63; H, 4.20; N, 5.28. $C_{13}H_{11}NO_2$ (261.2); requires: C, 59.77; H, 4.24; N, 5.36. The spectroscopic characterization is given in Table 1.

4-*Hydroxymethyl*-1-*methoxycarbonylazulene* (**11**). To a suspension of zinc powder (0.70 g) in HOAc (30 ml) a soln of **10**

(0.65 g, 2.49 mmol) in HOAc (5 ml) was added at 0° slowly and with vigorous stirring. After 2 hr at 0° the reaction mixture was filtered, the filtrate was diluted with H_2O (50 ml) and extracted with Et_2O (×3, 50 ml). The combined extracts were dried (Na_2SO_4), the solvent removed *in vacuo* and the crude product purified by CC (neutral Al_2O_3, activity grade I; eluent CH_2Cl_2); 0.44 g **11** (81%), dark-blue oil. Found: C, 72.09; H, 5.44; O, 22.01. $C_{13}H_{12}O_3$ (216.2); requires: C, 72.21; H, 5.59; O, 22.20. The spectroscopic characterization is given in Table 1.

1-*Methoxycarbonyl-4-methoxymethylazulene* (**12**). The hydroxymethyl ester **11** (0.40 g, 1.85 mmol) in MeOH (20 ml) was reacted with CH_2N_2 in Et_2O {prepared from *N*-nitrosomethylurea (1.00 g, 9.71 mmol) according to the procedure in ref. [13]}. After the usual workup and purification by CC (neutral Al_2O_3, activity grade III; eluent CH_2Cl_2) the product was obtained as blue-violet oil; 0.26 g **12** (61%). Found: C, 72.88; H, 6.00; O, 20.69. $C_{14}H_{14}O_3$ (230.2); requires: C, 73.03; H, 6.13; O, 20.84. The spectroscopic characterization is given in Table 1.

4-*Formyl-1-methoxycarbonylazulene* (**13**). The hydroxymethyl ester **11** (0.50 g, 2.31 mmol) was oxidized with PCC on Al_2O_3 (4.00 g ≈ 4.00 mmol) in CH_2Cl_2 (25 ml) according to the procedure in ref. [16]. After usual workup and purification by CC (neutral Al_2O_3, activity grade I; eluent CH_2Cl_2) the product was obtained as red-violet oil; 0.37 g **13** (75%). Found: C, 73.00; H, 4.82; O, 22.50. $C_{13}H_{10}O_3$ (214.2); requires: C, 72.89; H, 4.71; O, 22.41. The spectroscopic characterization is given in Table 1.

1,4-*Dimethyl-3-formylazulene* (**14**). 1,4-Dimethylazulene (**1**) (1.00 g, 6.40 mmol), dimethylformamide (20 ml) and $POCl_3$ (1.00 g, 6.52 mmol) were reacted according to the procedure in ref. [15]. After the usual workup and purification by CC (neutral Al_2O_3, activity grade I; eluent Et_2O) the product was obtained as violet crystals; 0.98 g **14** (83%), mp 73–74°. Found: C, 84.71; H, 6.50. $C_{13}H_{12}O$ (184.2); requires: C, 84.75; H, 6.57. The spectroscopic characterization is given in Table 1.

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