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BIOMIMETIC SYNTHESIS OF DALRUBONE AND OF A NEW PIGMENT FROM *DALEA EMORYI*

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Key Word Index—*Dalea emoryi*; Leguminosae; biomimetic synthesis; flavonoid; pigment.

The isolation of two unusual pigments from *Dalea emoryi*, dalrubone **2** and its 5-methoxy analog together with coumarin and 5-methoxycoumarin was recently reported by Dreyer *et al.* [1]. The structure of dalrubone and the co-occurrence of coumarin suggest that its biosynthesis in *Dalea* species may involve *C*- and *O*-methylation of flavylium salt intermediates such as **1**, although anthocyanidins or flavylium salts with this unusual oxygenation pattern have not yet been reported from any natural sources. In order to test this hypothesis we have prepared flavylium salt **1** employing a general synthetic method described by Michaelidis and Wizinger [2] and have methylated it under a variety of conditions.

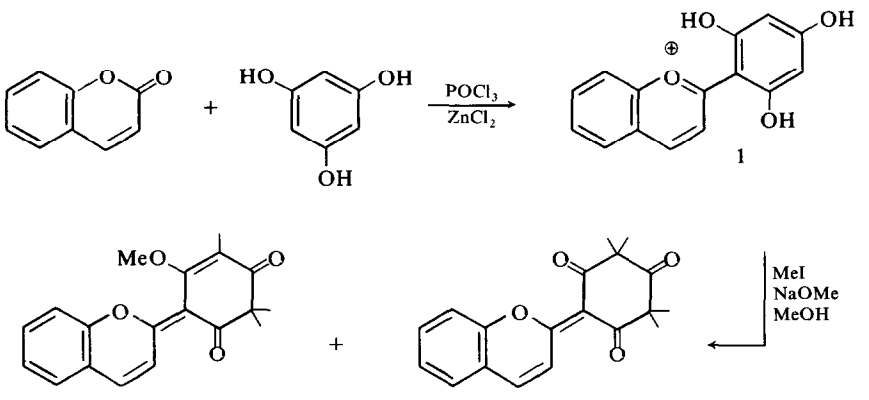
Methylation of **1** with MeI in refluxing methanolic NaOMe (mole ratio 1:10:6) gave a complex mixture of Et_2O-soluble products most of which were acidic and removed by extraction with aqueous base. Repeated column chromatography of the alkali-insoluble fraction yielded small amounts of two pure pigments.

The orange, less polar pigment possessed spectral properties (UV, MS, PMR, IR) and TLC behaviour in a number of solvent systems identical to those reported [1] for dalrubone, and this identity was confirmed by direct comparison with an authentic sample.

The yellow, more polar pigment has the molecular formula $C_{19}H_{18}O_4$ (MS), and 3 IR absorption bands in the carbonyl region at 1720, 1675 and 1636 cm^{-1} but no OH absorptions. The PMR spectrum in $CDCl_3$ was comprised of a pair of AB doublets at δ8.35 and 7.71 (J = 10 Hz) indicative of a *cis* disubstituted olefin, a 4 proton multiplet in the aromatic region at δ7.35–7.64, and a 12 proton singlet at δ1.39 caused by 4 equivalent Me groups. These data suggest structure **3** for the yellow pigment. This structural assignment was further confirmed by a study of the MS fragmentation pattern which can be rationalized as shown in Scheme 1. Additional support for the scheme was provided by a study of metastable peaks which were observed for all of the transformations shown except m/e 171 → 170.

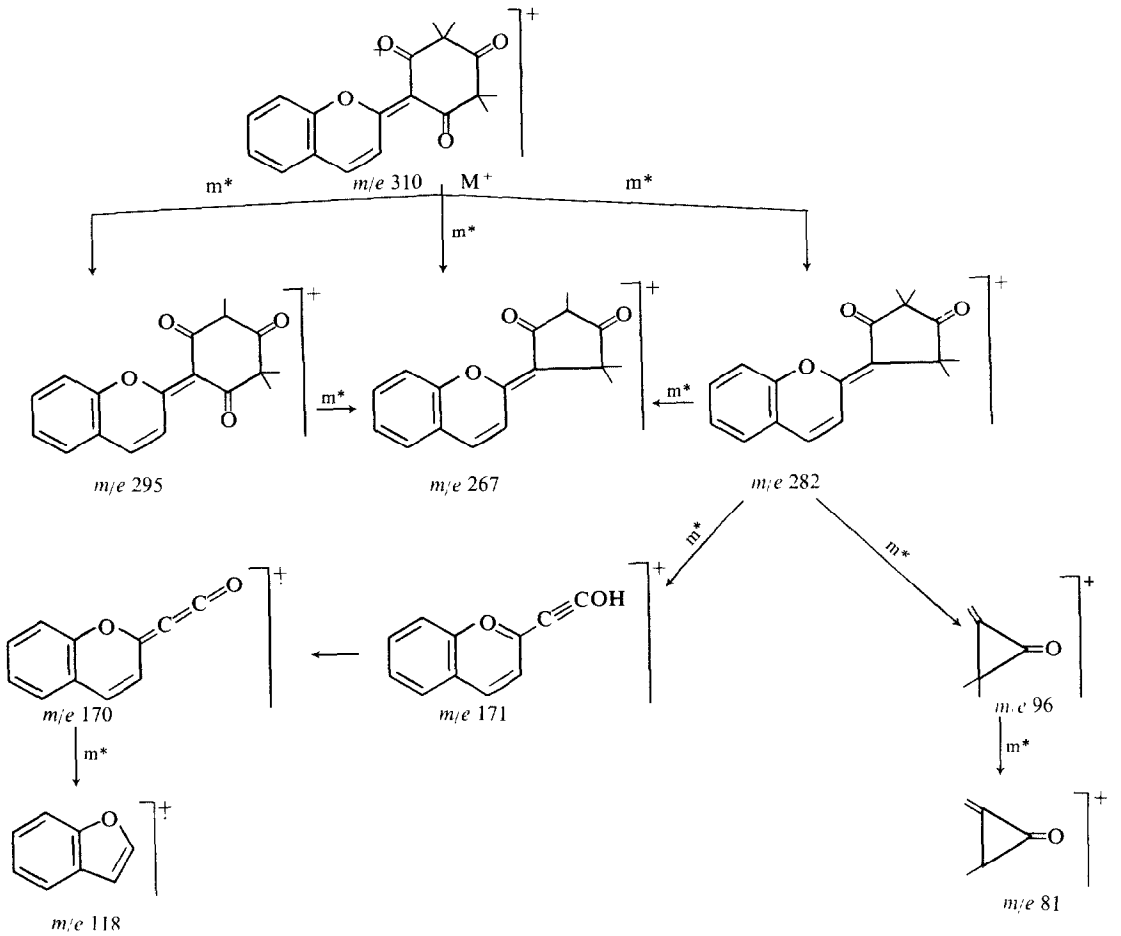
To further support the proposal that biosynthesis of *Dalea* pigments involves methylation of 2′-hydroxyflavylium intermediates, a sample of *D. emoryi* was extracted with C_6H_6 and after repreated chromatography on Si gel a yellow pigment of identical R_f to **3** was isolated in addition to dalrubone. The pure pigment for which we now propose the trivial name emorydone, proved to be identical in all respects (TLC, UV, PMR, MS, IR) with the synthetic methylation product **3**.

Because the majority of products from the methylation of **1** were acidic, further methylation of these was attempted. However, treatment with Et_2O–CH_2N_2, alkaline methanolic MeI, or Me_2SO_4 Me_2CO with K_2CO_3 failed to yield additional neutral products. Coupled with the TLC behaviour of these byproducts these observations strongly suggest that these materials are oligomeric. Methylations of **1** employing variations in time, temperature, and mole ratios of **1**, NaOMe and MeI did not succeed in supressing oligomer formation. Moreover, other types of methylation procedures were employed to no avail; among these were treatment of **1** with (a) MeI and BaO in DMF (b) Et_2O–CH_2N_2



2
Dalrubone

3
Emorydone



Scheme 1. Major mass spectral fragmentation routes of emorydone 3.

containing a little BF_3 etherate, and (c) MeI in refluxing liquid NH_3. Although methylation of flavylium salt 1 occurs without apparent control, the formation of two natural products during the reaction supports Dreyer's biosynthetic proposal.

EXPERIMENTAL

PMR spectra were obtained in $CDCl_3$ employing 40 pulses on a 99.5 MHz FT spectrophotometer and shifts are reported in ppm δ from internal TMS. EI MS were obtained at 70 eV.

Synthesis of 2-(2,4,6-trihydroxyphenyl)-benzopyrylium chloride (1). Phloroglucinol (12.6 g) was added to a mixture of coumarin (14.6 g) dry $ZnCl_2$ (15 g) and $POCl_3$ (50 ml) which had been warmed for 20 min at 100°. After an additional 1 hr warming, the mixture was cooled to 0° as 300 ml of 10% aq. $HClO_4$ was added, slowly at first. The scarlet needles which separated on standing were collected, washed with EtOAc and Et_2O, and dried (7.1 g). Recrystallization from dil. aq. $HClO_4$ provided the perchlorate salt of 1, mp 232°, $\lambda_{max}^{EtOH-HCl}$ 448 nm. (Found: C, 50.6; H, 3.25. $C_{15}H_{11}ClO_8$ requires C, 50.79; H, 3.13%).

Methylation of 1. A mixture of 1, (1 g), NaOMe (1.1 g), MeI (4.9 g), and MeOH (25 ml) was refluxed for 2 hr, the solvent removed by rotary evaporation, and the residue shaken with Et_2O and 2% aq. NaOH. The residue after removal of solvent from the Et_2O layer was chromatographed on Si gel (cyclohexane–EtOAc, 19:1) yielding orange and yellow pigment fractions. Separate repeated chromatography of these on Si gel yielded *ca* 1 mg each of pure orange pigment R_f 0.46 and pure yellow pigment R_f 0.28 (cyclohexane–EtOAc, 4:1). The orange pigment was identical to dalrubone 2 (IR, UV, MS, PMR) [1]. The yellow pigment had M^+ *m/e* 310.1196; $C_{19}H_{18}O_4$ requires 310.1205. UV: λ_{max}^{EtOH} nm (rel. abs.) 250 (0.54), 274 (0.56), 380 (infl.), 400 (infl.), 419 (0.84), 443 (infl.). IR: ν_{max}^{KBr} cm^{-1} 1720, 1675, 1636. PMR: δ8.35 (1H, *d*, *J* = 10 Hz, C-3 or C-4), δ7.71 (1H, *d*, *J* = 10 Hz, C-3 or C-4), δ7.35–7.64 4H, *m*, C-5, C-6, C-7, C-8), δ 1.39 (12H, *s*, 4 × Me). MS (probe) *m/e* (rel. int.): 310 [M^+] (100), 295 (23), 282 (12), *m** 280.9 (310 → 295), 267 (16), *m** 256.6 (310 → 282), *m** 252.8 (282 → 267), *m** 241.9 (295 → 267), 240 (12), *m** 230.2 (310 → 267), 215 (17), 184 (13), 171 (83), 170 (60), 118 (34), *m** 103.5 (282 → 171), 96 (47), *m** 81.9 (170 → 118), 81 (50), *m** 68.4 (96 → 81).

Isolation of emorydone 3. *D. emoryi* (100 g, collected in Baja California, Mexico by D. L. Dreyer) was successively extracted with petrol (2 days) and warm C_6H_6 (2 days). The gum (0.8 g) obtained on removal of solvent from the C_6H_6 extract was separated into orange and yellow pigment fractions by initial chromatography on Si gel (cyclohexane–EtOAc, 19:1). The orange material after rechromatography on Al_2O_3 yielded *ca* 1 mg of pure dalrubone 2. The yellow fraction after repeated chromatography on Si gel gave < 1 mg of pure emorydone 3 identical (TLC, UV, IR, MS, PMR) with the sample isolated from methylation of 1.

Acknowledgements—The authors wish to thank Dr. D. L. Dreyer for kindly providing an authentic sample of dalrubone as well as some unextracted *D. emoryi* plant. We also appreciate the aid of Dr. W. Haddon who obtained the MS and assisted in their interpretation.

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6-METHOXYKAEMPFEROL 3-*O*-GLUCOSIDE FROM *FLAVERIA BROWNII*

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Key Word Index—*Flaveria brownii*; Compositae; new flavonol glucoside; 6-methoxykaempferol 3-glucoside.

We report the isolation and structure determination of a new flavonol glycoside, the 3-*O*-glucoside of 6-methoxykaempferol, from the leaves and stems of *Flaveria brownii* collected in south Texas.

The mass spectrum of the perdeuteriomethyl ether of the glycoside gave an aglycone ion at *m/e* 367 (97% relative intensity to the base peak) as expected for the loss of the C_3-*O*-glycosyl moiety and the introduction of three deuteriomethyl groups at the 5,7 and 4′ positions on a 6-methoxykaempferol skeleton. Other prominent peaks were *m/e* 368 (40%), 366 (10%) and 352 (60%); this latter peak is typical for 6-methoxyflavonols. The sugar obtained by 2N HCl hydrolysis of the natural product was identified as glucose by co-chromatography on PC and by GLC of its trimethylsilyl ether. The aglucone appeared yellow-green when viewed on paper over UV light, typical for a flavonol. Moreover, the aglycone was identical with an authentic sample of 6-methoxykaempferol [1] by co-chromatography in three different systems.

The NMR spectrum (in CCl_4) of the trimethysilyl ether of the natural product gave typical kaempferol B-ring proton signals: two doublets (J = 9Hz) at δ 7.9 for H-2′ and H-6′ and at δ 6.86 for H-3′ and H-5′. Other aromatic signals included a singlet at δ 6.45 typical for an isolated proton at C-8, and a sharp three-proton methoxy singlet at δ 3.65. The latter signal shifted upfield only 0.07 ppm in benzene in accord with a 6-methoxyl group. A one-proton doublet (J = 5Hz) at δ 5.8 could be assigned to the H-1 proton in a C_3-*O*-glucosyl moiety; six other glucosyl protons appeared between 3.3 and 3.58 ppm. The UV spectrum of the natural product in MeOH exhibited Band I at 338 nm and this combined with the absence of a shoulder on Band II supported a kaempferol-type B-ring. The Band I shift of 64 nm with an increase in intensity for the NaOMe spectrum is in accord with a 4′-hydroxyl group. The 24 and 21 nm shifts of Band I in $AlCl_3$ and $AlCl_3$/HCl, respectively (both relative to Band I in MeOH) are in the range for a 6-methoxyl group in a C_5-OH, C_3-*O*-substituted flavonol [2]. The shoulder at 398 nm on Band I in $AlCl_3$/HCl is also in accord with the presence of a 6-methoxyl group [2]. The presence of Band III in the NaOMe spectrum at 330 nm and Band I in NaOAc appearing at shorter wavelength relative to Band I in NaOMe are diagnostic for a free 7-hydroxyl group [3]. Since the aglucone is 6-methoxykaempferol, the above data establish a 3-*O*-glucosyl group; thus, the natural product is 6-methoxykaempferol 3-*O*-glucoside, a new compound from nature.

EXPERIMENTAL

Air dried leaves and stems of *Flaveria brownii* (collected at Port Aransas, Texas; a voucher specimen, Powell 2802, is deposited in LL Herbarium, The University of Texas at Austin) were ground to a fine powder, which was extracted at room temp. with a 85% aq. MeOH for 24 hr. The extract was filtered and concd *in vacuo*, then extracted with $CHCl_3$ followed by EtOAc. The EtOAc fraction was chromatographed over polyamide packed in MeOH; 6-methoxykaempferol 3-*O*-glucoside was eluted with MeOH in the first fractions: R_f values 0.66 (TBA); 0.54 (15% HOAC); UV: λ_{max}^{MeOH} 271, 293, 338 nm; NaOMe: 281, 330, 402 (no dec.) nm; $AlCl_3$: 278, 301 *sh*, 362, 392 *sh*; $AlCl_3$/HCl: 281, 307 *sh*, 359, 398 *sh*; NaOAc: 273, 312 *sh*, 336, 396; NaOAc/H_3BO_3: 270, 346. Acid hydrolysis of the glycoside afforded glucose and 6-methoxykaempferol. The aglycone was identical with an authentic sample by polyamide TLC, $CHCl_3$–MeOH–MeCOEt–Me_2CO (10:10:5:1) R_f 0.72; PC, TBA and 50% HOAc, R_f 0.80 and 0.62, respectively.

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