not carry the biflavonoid linkage. The ion at m/z 281 (29%) corresponding to the crucial fragment (**9**) is formed after two RDA fragmentations. Compound **6** showed a peak at m/z 566 (8%), which is formed by the loss of 46 mass units. This fragment can be formulated as **10** in which the *ortho*-methoxyl groups to the biphenyl linkage cyclize to a furan ring [5]. Perhaps the most characteristic feature is the formation of the two fragments **9** and **10**, which could be indicative of a C–C linkage. There are two more ions at m/z 432 (10%) and 461 (11%) corresponding to **11** and **12**, respectively. It may be mentioned here that similar fragments have been reported in tetrahydroamentoflavone [6] and (±)-fukugetin heptamethyl ether [7]. Overall, the mass spectral fragmentation exhibits a close similarity to that of 2,3-dihydroamentoflavone hexamethyl ether [8] and GB-2 [9, 10].

From the foregoing spectral and chemical studies, structure **5** has been assigned for semecarpuflavanone with the interflavonoid linkage at the B-3′–D-8″ position.

EXPERIMENTAL

Oxidation of semecarpuflavanone (**5**) *with neutral permanganate.* A mixture of **5** (100 mg), $KMnO_4$ (100 mg) in dry Me_2CO (20 ml) was left at room temp. for 1 hr. The solvent was then evaporated and diluted with H_2O. The manganous salts were decomposed with SO_2 and extracted with Et_2O. The Et_2O extract was shaken with 1% aq. $NaHCO_3$, acidified with dilute HCl and again extracted with Et_2O, dried and evaporated. The residue on crystallization from H_2O gave needles, mp 250–251° (decomp.), yield: 14 mg; identical with authentic gallic acid (mmp and IR). As the theoretical yield, calculated on the basis of structure **5** (molecular formula, $C_{30}H_{22}O_{10}$), is 31.36 mg, it can be easily seen that 1 mol of the acid resulted in this oxidation.

Oxidation of semecarpuflavanone pentamethyl ether (**6**) *with neutral permanganate.* Compound **6** (100 mg) was oxidized with $KMnO_4$ (100 mg) in dry Me_2CO (20 ml) for 1 hr and worked-up as above. The residue was extracted with hot C_6H_6 and concd resulting in a crystalline solid, mp 204°, yield: 12 mg; identical with authentic syringic acid (mmp and IR). The residual solid was crystallized from MeOH giving a colourless solid, mp 155–156°, yield: 10 mg; identical with authentic 2-hydroxy-4-methoxybenzoic acid (mmp and IR). Compound **6**, on methoxyl estimation (Zeisel's method), gave 4.96 as the methoxyl value, which indicated that only five methoxyls are present.

Acknowledgements—I am grateful to Professor L. R. Row for encouragement. My thanks are also due to Drs. P. A. Ramaiah and M. Bapuji for recording the ^1H NMR and mass spectra.

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Phytochemistry, Vol. 22, No. 6, pp. 1520–1521, 1983.
Printed in Great Britain.
0031-9422/83/061520-02$03.00/0

FLAVONOIDS IN THE SEEDS OF *ARGEMONE MEXICANA*: A REAPPRAISAL

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(*Received* 22 *November* 1982)

Key Word Index—*Argemone mexicana*; Papaveraceae; seeds; flavonoids; luteolin; eriodictyol.

Abstract—Re-examination of the seed extract of *Argemone mexicana* for the newly reported 5,7,2′,6′-tetrahydroxyflavone failed to indicate the presence of any novel flavone. The major seed flavone is, in fact, luteolin and this is accompanied by the related flavanone, eriodictyol. The danger of relying entirely on spectral measurements for identifying new flavonoids is highlighted by these findings.

In the process of identifying a novel tetrahydroxyflavone in some *Lupinus* species, we were interested in obtaining a sample of 5,7,2′,6′-tetrahydroxyflavone for comparative purposes. This substance has recently been reported as a new natural flavone, independently, from *Argemone mexicana* seed [1] and from *Scutellaria baicalensis* root [2].

The reported properties of this new flavone differ so much that one or other of these two reports must be incorrect; for example the UV spectral maxima in methanol are reported as 270 and 343 nm in one paper [1] and as 261 and 310 nm in the other [2]. We now wish to report our findings that the major seed flavone of *Argemone mexicana* is not a new compound but it is the well-known isomeric structure, luteolin or 5,7,3',4'-tetrahydroxyflavone.

Simple 80% methanolic extraction of a small seed sample of authentic *A. mexicana* gave an extract, which on 2D-PC showed the presence of a single major flavone (R_fs 0.70 in BAW, 0.06 in 15% HOAc) together with a major dark-absorbing compound (R_fs 0.87 in BAW 0.53 in 15% HOAc) of a flavonoid type. The flavone was isolated and purified. It was clearly identical to luteolin in all its spectral properties (and shifts) and this was confirmed by co-chromatography with an authentic marker in seven solvents. In addition, a study of the mass spectrum, which was not undertaken by the Indian workers [1], showed the characteristic fragmentation pattern of luteolin, with a molecular ion at 286 a.m.u. and peaks at 258 $[M-28]^+$, at 153, 124 and 123 (dihydroxy A-ring) and at 109 a.m.u. (catechol B-ring). According to the literature [3], 2'-hydroxyflavones show a significant peak at $[M-17]^+$ but no such peak could be detected in our sample. Moreover, our flavone in the UV spectrum showed the expected borate shift typical of 3',4'-disubstitution; the Indian authors failed to carry out this test. Their only evidence for the lack of a catechol B-ring was the absence of a colour with ammonium molybdate, but such colour tests are notoriously unreliable as negative evidence. The authors' only positive evidence for 2',6'-disubstitution was based on the ^{1}H NMR spectra of the tetramethyl ether and tetra-acetate but here, without careful comparison with model compounds, assignments can be made in error.

The other major flavonoid component in *A. mexicana* seed was isolated by PC and readily identified as the flavanone eriodictyol (5,7,3',4'-tetrahydroxyflavanone) by direct comparison with an authentic sample.

The Indian authors [1] also reported the presence in the seed of 5,7-dihydroxychromone 7-neohesperidoside. We were not able directly to either confirm or refute this finding. However, we would note that earlier reports of the natural occurrence of hydroxychromones unsubstituted at position-2 have been brought into question through the experiments of Stocker and Pohl [4] who found that chromones are rather readily formed by aerial oxidation from structurally related flavanones through the rupture of the 2–1' carbon–carbon bond. Such an oxidation could occur during a lengthy isolation process, as on a Si gel column. In the case of the above report, this would require the presence in the seeds of a flavanone 7-neohesperidoside, such as naringin or eriodictyol 7-neohesperidoside. Our chromatogram of the seed extract showed the presence of two components (R_fs 0.67 in BAW, 0.40 in 15% HOAc; 0.42 in BAW, 0.28 in 15% HOAc) with the colour reactions of flavanone glycosides. It would, therefore, appear that, in this case too, it is possible that this chromone is an artifact of the isolation procedure.

The discrepancy between our findings and those of Bhardwaj *et al.* [1] on the major seed flavone of *Argemone mexicana* is very unlikely to be due to misidentification of plant material since this plant is a well-known, highly distinctive weed species. It could be due to the presence of different chemical races. However, this possibility was largely ruled out when we found the same two flavonoid constituents, luteolin and eriodictyol, in four other seed accessions.

We conclude, therefore, that *Argemone mexicana* seed does not contain 5,7,2',6'-tetrahydroxyflavone and the trivial name argemixitin proposed for it should be abandoned. The report of this new flavone in *Scutellaria baicalensis* root seems to be more secure, since it is based on both ^{1}H NMR and ^{13}C NMR spectral studies. We are in the process of re-isolating the flavones from this source for purposes of confirmation.

The present results indicate the danger of relying too heavily on spectral data for structural assignments in the flavonoid series. In this case, chromatographic comparison with known markers would have immediately revealed the identity of the *Argemone* flavone. Additionally, the incorrectness of the analysis would have been apparent if a comparison with a synthetic sample had been attempted. The use of either chemical degradation or chemical synthesis would seem to be an essential prerequisite before reporting the presence in a plant of a novel flavonoid aglycone.

EXPERIMENTAL

Plant material. The main accession of *Argemone mexicana* L. seed examined was supplied by the Royal Botanic Gardens, Kew, under the accession No. 000–69–19957. Other seed samples were obtained from the Oxford Botanic Garden, Chelsea Physic Garden and Thompson & Morgan.

Flavonoid identifications. Luteolin from *A. mexicana* seed had UVλ_{max}^{MeOH} nm: 255, 267, 350; +NaOAc: 257 sh, 276, 360; +NaOAc–HBO_3:260, 372. It co-chromatographed with an authentic sample by TLC on microcrystalline cellulose in BAW, Forestal, 50% HOAc and CAW; on polyamide in toluene–MeCOEt–MeOH (4:3:3); and on Si gel in $CHCl_3$–MeOH–H_2O–HOAc (100:15:0.5:0.3) and in toluene–HCO_2Et–HCO_2H (5:5:1). R_fs in the latter two solvents were 0.27 and 0.51, respectively, and thus luteolin seems to be clearly separable from genuine 5,7,2',6'-tetrahydroxyflavone, as reported in *Scutellaria* roots, where R_fs of 0.41 and 0.65, respectively, were measured [2]. The amount of luteolin was determined in a separate expt as 2.4 mg/g of dry seed.

Eriodictyol from *A. mexicana* seed had UVλ_{max}^{MeOH} nm: 287, sh, 320; +NaOH: 324. It cochromatographed with an authentic sample by TLC on microcrystalline cellulose in PhOH, CAW, 50% HOAc, 15% HOAc and BAW.

Acknowledgement—We are grateful to Mr. R. Butters of Tate & Lyle Research Laboratories for mass spectral measurements.

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