Phytochemistry, Vol. 21, No. 9, pp. 2428–2429, 1982.
Printed in Great Britain.

0031-9422/82/092428-02$03.00/0

APIGENINIDIN AS A LEUCODERIVATIVE IN *EPHEDRA FRUSTILLATA*

ALBERTO A. GURNI and MARCELO L. WAGNER

Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Botánica y Museo Botánico Juan A. Dominguez, Buenos Aires, Argentina

(*Received* 17 *November* 1982)

Key Word Index—*Ephedra frustillata*; Ephedraceae; apigeninidin; pelargonidin; leucoderivatives.

Abstract—Aerial parts of *Ephedra frustillata* are shown to contain leucoderivatives based on apigeninidin and pelargonidin. This is the first report of leucoapigeninidin in gymnosperms.

The present study is a contribution to the phytochemistry of the Argentinian species of *Ephedra*. Previous studies have been concerned with the determination of the presence of the protoalkaloid ephedrine and of tannins [1, 3] and with phytochemical screening [2]. More recently, vicenin I and II have been reported from *E. andina* [4].

In the present study stems showing secondary growth of *E. frustillata* Miers were studied. Their extracts were colourless but developed an anthocyanin colouration after acid treatment. Chromatographic separation in three solvents led to red and yellow–orange spots. The red spot turned blue after fuming with ammonia and was found to correspond to pelargonidin, while the yellow–orange one turned red after the same treatment and was shown to represent the 3-desoxy-anthocyanidin apigeninidin. By heating the chromatograms with 2 N hydrochloric acid the two anthocyaninidins were generated on the paper. Thus it is assumed that the two anthocyaninidins are present in the tissue as leucoderivatives.

While a condensed leucopelargonidin has been recorded already from several Asian species of *Ephedra* [14], this is the first report of the leucoderivative giving rise to apigeninidin in gymnosperms. Up to the present, 3-desoxy leucoderivatives have been found only in six grass species of the tribe Andropogoneae [11, 12] and in *Zea mays* [13]. Glycosides of 3-desoxyanthocyanidins appear to be more widespread and have been found in 1 moss [5] in 9 forms, 8 species (6 genera and 3 families) of the monocotyledons [6, 7–9] and in 22 species (15 genera and 5 families) of the dicotyledons [6, 10], of which the majority (19 spp.) belong to the Gesneriaceae.

The occurrence of leucoderivatives of anthocyanidins in *E. frustillata* may indicate that these substances are present in the native tissue (1) in the form of condensed compounds (proanthocyanidins), or (2) as colourless precursors of low micro MW. Work is continuing in order to answer this question.

EXPERIMENTAL

Plant material was collected by A. A. Gurni in Argentinian Patagonia. Identification was made by Drs. Sánchez and Caro on the basis of the revision of Argentinian *Ephedra* [15] and by means of anatomical studies [16]. Voucher specimens are deposited in Museo Botánico Juan A. Dominguez of the Faculty for Pharmacy and Biochemistry, University of Buenos Aires. Dried stems of *E. frustillata* were powdered and extracted with boiling H_2O. Extracts were evaporated under red. pres. and the residues dissolved in MeOH. These MeOH solns were treated with 2 N HCl at 100° for 1 hr. After cooling they were shaken with a small vol. of amyl alcohol. These amyl alcohol extracts were chromatographed in formic (HCOOH–HOAc–H_2O, 9:2:3) on Whatman paper 3 MM. The separated pigments were then eluted with 1% HCl in MeOH and chromatographed in formic, BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper layer) and Forestal (HCl–HOAc–H_2O, 3:30:10) [6] on Whatman paper No. 1 together with authentic samples. MeOH plant extracts were then chromatographed on Whatman paper No. 1. First dimension: *n*-BuOH–HOAc–H_2O, 14:1:5M upper layer; second dimension: 6% HOAc. This is a slight modification of the solvents used by Haslam [17] who used *s*-BuOH. After drying the chromatograms, 2 N HCl was sprayed on them and they were placed in an oven at 100° for 15 min. After this treatment apigeninidin and pelargonidin were visible. R_f values of the colourless precursor of apigeninidin 0.72 (first solvent), 0.13 (second solvent), of pelargonidin 0.72 (first solvent). 0.34 (second solvent). Pigment identification was confirmed by spectral measurements.

Acknowledgements—We are grateful to Professor J. B. Harborne, University of Reading, England, for providing a sample of apigeninidin; to Professor K. Kubitzki, University of Hamburg, Germany, for encouragement, and to Proffessor J. A. Caro and Dra. E. A. Sánchez, Buenos Aires, for identifying the specimens.

REFERENCES

1. Calastremé Cortejarena, A. M. (1951) *Rev. Invest. Agríc.* **5**, 375.
2. Gurni, A. A. and Moreira J. D. (1974) *Darwiniana* **18**, 520.
3. Diez, V. (1938) Doctoral thesis, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.
4. Castledine, R. M. and Harborne, J. B. (1976) *Phytochemistry* **15**, 803.
5. Bendz, O., Martensson, O. and Terenius, L. (1962) *Acta Chem. Scand.* **16**, 1183.
6. Harborne, J. B. (1966) *Phytochemistry* **5**, 589.

7. Clifford, H. T. and Harborne, J. B. (1969) *Phytochemistry* **8**, 123.
8. Misra, K. and Seshadri T. R. (1976) *Indian J. Chem.* **5**, 409.
9. Singh, N. S. (1972) *Phytochemistry* **11**, 163.
10. Meditsch, J. de O. and Barros, E. C. 1978 *An. Assoc. Bras. Quim.* **29**, 89.
11. Bate-Smith, E. C. and Swain, T. (1967) *Nature (London)* 213, 1033.
12. Kambal, A. E. and Bate-Smith, E. C. (1976) *Heredity* **37**, 413.
13. Styles, E. D. and Ceska, O. (1972) *Phytochemistry* **11**, 3019.
14. Taraskina, K. V., Chumbalov, T. K., Chekmeneva L. N. and Chukenova, T. (1974) *Chem. Abstr.*, 166323e. **81**.
15. Hunziker, J. H. (1969) *Lilloa* **17**, 147.
16. Sánchez, E. and Caro, J. A. (1974) *Darwiniana* **18**, 511.
17. Haslam, E. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds.), p. 536. Chapman & Hall, London.

Phytochemistry, Vol. 21, No. 9, pp. 2429–2430, 1982.
Printed in Great Britain.

0031–9422/82/092429–02$03.00/0

AN ISOFLAVONE GLYCOSIDE FROM THE HEARTWOOD OF *PTEROCARPUS MARSUPIUM*

J. MITRA and T. JOSHI

Department of Chemistry, University of Allahabad, Allahabad, 211 002, India

(*Received* 17 *July* 1981)

Key Word Index—*Pterocarpus marsupium*: Leguminosae; 5,4′-dimethoxy-8-methylisoflavone 7-*O*-α-L-rhamnopyranoside.

Abstract—A new isoflavone, 5,4′-dimethoxy-8-methylisoflavone, has been identified from the heartwood of *Pterocarpus marsupium*.

Species of *Pterocarpus* are known to be rich in isoflavonoids and terpenoid derivatives [1]. From the ethyl acetate fraction of the ethanolic extract of the heartwood of *P. marsupium* a new isoflavone glycoside has been identified.

The compound had a molecular formula $C_{24}H_{26}O_9$, mp 185°, (*d*) gave the characteristic colour reactions of an isoflavone and was found to be glycosidic in nature. Its isoflavone nature was further confirmed by its UV and ^{1}H NMR spectra. Hydrolysis with 7% sulphuric acid gave rhamnose (co-chromatography with an authentic sample) and a yellow aglycone, $C_{18}H_{16}O_5$, mp 195°. The aglycone analysed for one hydroxyl (acetate and IR 3350 cm^{-1}), one *C*-methyl group (^{1}H NMR signal at δ 2.3 corresponding to three protons of CH–Me) and two methoxyl groups (Ziesel, IR ν_{max} cm^{-1} : 2865, 1185 and ^{1}H NMR signal at δ 3.8 corresponding to 6H of 2-OMe).

Spectral studies of the aglycone (UV λ_{max} nm: 260, 316(sh) indicated the presence of one free hydroxyl at position 7 (bathochromic shift of 12 nm of band II with fused sodium acetate and 8 and 10 nm shifts of band II and band I, respectively with sodium methoxide). In order to assign the positions of the methyl and methoxyl groups the aglycone was methylated with diazomethane and the resulting methyl ether subjected to alkali fission. One of the products was identified as the 2,4-dimethyl ether of 6-hydroxytoluene mp 60° (lit. 61°) [2]. Formation of this product confirms the position of one methyl group at position 8 and a methoxyl at both the 5- and 7-positions on the A ring. The other product formed was *p*-methoxyphenylacetic acid mp 83° (lit. 84°) and this clearly indicates the presence of one methoxyl group at the 4′-position of the B ring. The structure is further confirmed by 2′, 6′-proton signals at δ 7.4 and 3′, 5′-proton signals at δ 6.6 in the ^{1}H NMR spectrum. ^{1}H NMR also showed a singlet at δ 6.4 (C-6 proton of ring A) and confirmed that the 5-, 7- and 8-positions of ring A are substituted. A sharp signal at δ 7.8 corresponding to the C–2 proton of ring C which is specific for isoflavones, was also present in the ^{1}H NMR spectrum [3]. Thus the aglycone is 7-hydroxy-5,4′-dimethoxy-8-methylisoflavone. The attachment of the sugar molecule was confirmed from comparison of the UV spectra of the aglycone and glycoside. The aglycone gave a 12 nm bathochromic shift with sodium acetate whilst the glycoside gave no shift suggesting that the rhamnose is attached at the 7-position. Rhamnose is in the pyranose form since periodate oxidation gave 2 mol periodate per mol glycoside consumed and 1 mol formic acid was produced. The glycoside was hydrolysed by takadiastase but not by emulsin showing the presence of an α-linkage.

The isoflavone glycoside is thus 5,4′-dimethoxy-8-