Phytochemistry, 1980 Vol. 19, pp. 2229–2230 © Pergamon Press Ltd. Printed in England. 0031-9422/80/1001-2229 $02.00/0

UNUSUAL FLAVONES FROM *OCIMUM CANUM*

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(*Received* 26 *November* 1979)

Key Word Index—*Ocimum canum*; Labiatae; flavones; nevadensin; salvigenin; triterpene acids.

Ocimum canum Sims (Somali name 'reexaan') is one of the several species of the genus *Ocimum* growing in Somalia; it is used for flavouring foods and for traditional medicine. No previous research has been reported on this species, and the genus has not been investigated widely for higher terpenoids. For these reasons, we examined the combined leaves and flowers of *O. canum* and report the occurrence of the flavones nevadensin and salvigenin, and ursolic and oleanolic acids.

Nevadensin (5,7-dihydroxy-6,8,4′-trimethoxyflavone) was characterized by comparison of MS, NMR, IR and UV data with an authentic marker. The identification was confirmed by its methylation to tangeretin (5,6,7,8,4′-pentamethoxyflavone) and by acetylation to diacetylnevadensin. The second flavone showed MS, NMR, IR and UV data in full agreement with those of salvigenin (5-hydroxy-6,7,4′-trimethoxyflavone). Identification was confirmed by methylation to tetra-*O*-methylscutellarein (5,6,7,4′-tetramethoxyflavone) and by acetylation to acetylsalvigenin.

Both nevadensin and salvigenin are rare flavones. Nevadensin has been found only three times in nature [1–3] and has not been reported previously from the Labiatae. Salvigenin occurs in some *Salvia* species [4–6] and in a number of other plants, *inter alia* [7–9].

The triterpene fraction contained only acids; after diazomethane treatment, the mixture of methyl esters was examined by GLC and found to contain methyl ursolate (90%) and methyl oleanolate (10%); no other peak was detected.

EXPERIMENTAL

Extraction and purification. The plant material was collected in May 1978 near Gonderscia (district of Merka) and was identified by Dr. F. M. Raimondo (Botanic Garden, University of Palermo; formerly, National Somali University, Faculty of Veterinary Sciences, Mogadishu, Somalia), Dr. I. C. Hedge (Royal Botanic Garden, Edinburgh, U.K.), and Dr. S. M. A. Kazmi (National Herbarium, National Range Agency, Mogadishu, Somalia). Specimens are deposited in the Herbaria under the number 'Mog 1 + 2 Uarsheekh'. Air-dried leaves and flowers (0.5 kg) were pulverized and extracted twice with Me_2CO (2 l.) at room temp. for 3 days. The combined extracts were evapd to dryness and the residue taken up in cold EtOAc (500 ml) for 24 hr; a yellow crystalline product was separated (crude nevadensin, 3 g), the solvent evapd and the residue (50 g) chromatographed on Si gel (800 g) deactivated with 15% H_2O using the dry-column technique. Petrol eluted essential oils, fats and waxes. Elution with petrol–EtOAc (3:1) gave a mixture of ursolic and oleanolic acid (1 g), salvigenin (0.5 g) and nevadensin (0.5 g).

Identification of triterpene acids. A sample of the above mixture was methylated with CH_2N_2 and subjected to GLC (Varian Aerograph 1440, FID, 90 cm column packed with 3% OV-1 coated Chromosorb, temp. 260° carrier gas N_2 20 ml/min: two peaks after 6 min 30 sec and 7 min 5 sec (intensity 9:1) co-chromatographed with authentic samples of methyl ursolate and methyl oleanolate.

Nevadensin. Mp 193–194° (from EtOAc or Me_2CO). (Found: C, 62.76; H, 4.76. $C_{18}H_{16}O_7$ requires: C, 62.79; H, 4.68%). MS *m/e* (rel. int.): 344 (M, 68%), 329 (100), 197 (25), 169 (32), 135 (10), 132 (10). IR (nujol) cm^{-1}: 3400, 1605, 1590, 825. ^1H NMR (60 MHz, $CDCl_3$): δ 3.90–4.01–4.03 (*s*, 3 OMe), 6.55 (*s*, H-3), 7.02 and 7.84 (*dd*, $J = 9$ Hz, A_2B_2 system for 4 Ar-H), 10.40 (*br*, phenolic OH), 12.32 (*s*, chelated phenolic OH); (60 MHz, C_6D_6): δ 3.23–3.81–3.83 (*s*, 3 OMe). UV (abs. EtOH, *c* 7.96 mg/l): λ_{max} nm (ε): 280 (28 000) and 336 (11 000); with $AlCl_3$ λ_{max} 307 (24 500) and 348 (22 000); with fused NaOAc λ_{max} 280 (30 500) and 378 (11 600). Methylation with Me_2SO_4 and K_2CO_3 in Me_2CO gave tangeretin, mp 154°. Acetylation with Ac_2O and fused NaOAc gave diacetylnevadensin, mp 172–174°.

Salvigenin. Mp 188–189° (from EtOAc). (Found: C, 65.73; H, 4.82. $C_{18}H_{16}O_6$ requires: C, 65.85; H, 4.91%). MS *m/e* (rel. int.): 328 (M, 100%), 313 (91), 181 (28), 153 (78), 135 (28), 132 (35). IR (nujol) cm^{-1}: 1630, 1600, 830. ^1H NMR (60 MHz, $CDCl_3$): δ 3.87–3.93–3.96 (*s*, 3 OMe), 6.50 (*s*, H-3), 6.53 (*s*, H-8), 7.00 and 7.82 (*dd*, $J = 9$ Hz, A_2B_2 system for 4 Ar-H), 12.70 (*s*, chelated phenolic OH); (60 MHz, C_6D_6): δ 3.27–3.30–3.92 (*s*, 3 OMe). UV (abs. EtOH, *c* 8.68 mg/l): λ_{max} nm (ε) 275 (20 200) and 328 (26 300); with $AlCl_3$ λ_{max} 298 (21 600) and 348 (26 400); with fused NaOAc λ_{max} 275 (20 200) and 328 (25 900). Methylation gave tetra-*O*-methylscutellarein, mp 160–161°. Acetylation with Ac_2O and fused NaOAc gave acetylsalvigenin, mp 167–168°.

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Phytochemistry, 1980, Vol. 19, pp 2230–2231 © Pergamon Press Ltd. Printed in England. 0031 9422/80/1001-2230 $02.00/0

THE UBIQUITY OF CYCASIN IN CYCADS

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(*Received* 15 *December* 1979)

Key Word Index—Cycadales; cycads; Gymnospermae; chemotaxonomy; cycasin; methylazoxymethanol glycosides.

Cycadales, represented today by ten genera found in all continents except Europe, together with Ginkgoales are included in the prephanerogams, a relict group of ancient gymnosperms. The presence of glycosides of MAM (methylazoxymethanol) has been reported only in the seeds of some cycads and in smaller quantity in their stems and fronds.

Cycasin is the most abundant glycoside found in *Cycas revoluta* [1, 2] and *C. circinnalis* [3]; other MAM glycosides (neocycasins) occur in these plants but only in small quantities [2]. Macrozamin is a MAM glycoside found in *Macrozamia spiralis* [4] and *M. riedlei* [5]; it is probably also present in *M. moorei*, *M. pauli-guilielmi*, *M. hopei* (= *Lepidozamia hopei*), *M. douglasii* and *Bowenia spectabilis* [6].

In this work 17 species belonging to the ten genera of cycads have been analyzed for the presence of cycasin. Simultaneously, analyses were made of *Ginkgo biloba* (Ginkgoales), *Pinus canariensis* (Pinales), *Cephalotaxus harringtonia* (Cephalotaxales), *Araucaria cookii* (Araucariales) and *Marattia salicina* (Marattiales), representatives of gymnosperms and ferns related to cycads. Ripe seeds were usually used for analysis; in *Ceratozamia mexicana* and *Encephalartos umbeluziensis*, however, unpollinated ovules were examined, and in *Microcycas calocoma* and *Marattia salicina* fronds. Cycasin is present in all the cycad species examined in quantities varying between 0.01 and 0.72% (see Table 1). In *Marattia salicina* and in the other gymnosperms examined, cycasin is absent.

From the quantitative viewpoint, our results are not completely representative, since some of the material examined was not of wild provenance. However, the cycasin percentage found in *Cycas revoluta* seeds (0.28%), coming from specimens grown in Naples Botanical Garden (Italy), is similar to values reported by Nishida *et al.* (0.28%) [1] and by Nagahama (0.296%) [2], for specimens of the same species growing in the field.

Our results show that cycasin is characteristic of and exclusive to the cycads, being present in all ten genera of this group. It is absent from other gymnosperm taxa and from the fern *Marattia salicina*. These results are of ecological interest in that the seeds of cycads are often eaten and cycasin is carcinogenic and neurotoxic [7]. It is destroyed only if the seeds are repeatedly washed and soaked, a procedure which probably liberates and activates the emulsin present in the seeds [8].

EXPERIMENTAL

Materials. Seeds of *Cycas revoluta*, *Stangeria eriopus*, *Pinus canariensis* C. Sm. and *Cephalotaxus harringtonia* C. Koch, ovules of *Ceratozamia mexicana* and *Encephalartos umbeluziensis* and fronds of *Microcycas calocoma* and *Marattia salicina* Smith come from specimens grown in Naples Botanical Garden (Italy); seeds of *Cycas lane-poolei*, *Lepidozamia peroffskyana*, *Macrozamia diplomera*, *M. heteromera* and *M. moorei* were collected in the field and supplied by the Terrara firm (Gilgandra, Australia); seeds of *Bowenia spectabilis* were collected in the field and supplied by Mr. Brigden (Casuarina, Australia); seeds of *Encephalartos altensteinii* and *Zamia integrifolia* come from the cycad collection of Professor Verga (Como, Italy); seeds of *Dioon califanoi*, *D. edule*, *D. purpusii*