quercetin, kampferol and cyanidin (from leucocyanidin) have been found in the hydrolysed leaf extract of *C. religiosum* [1].

Present work. Extraction and identification of the flavonoids from the bark. The crude flavonoids were obtained by extraction of the milled bark with ethanol in the usual manner [2] and purified by means of column chromatography and preparative TLC. Apigenin (5,7,4′-trihydroxyflavone), naringenin (5,7,4′-trihydroxyflavanone) and (+)-afzelechin (5,7,4′-trihydroxyflavan-3-ol) were identified by NMR, IR, mmp and CoTLC and derivative formation [3–6]. The glycosides prunin (naringenin 7-*O*-glucoside) and cosmosiin (apigenin 7-*O*-glucoside) were identified by their physical data [6,7] and hydrolytic conversion to glucose together with naringenin and apigenin respectively.

Acknowledgements—Dr. J. W. W. Morgan for a sample of naringenin, Dr. R. W. Retallack for a sample of apigenin, and Professor J. W. Clark-Lewis for a sample of (−)-epiafzelechin.

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Phytochemistry, 1975, Vol. 14, pp. 2511–2512. Pergamon Press. Printed in England.

ACHILLIN AND DEACETYLMATRICARIN FROM TWO *ARTEMISIA* SPECIES

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(*Received* 25 *February* 1975)

Key Word Index—*Artemisia ludoviciana*; *A. klotzchiana*; Compositae; essential oil; camphor; borneol; achillin; deacetylmatricarin; 3,5-dihydroxy-6,7,8-trimethoxyflavone.

Plants. Artemisia ludoviciana, Nutt (Voucher specimen 7294) collected in Punta de la Loma, N.L.; *A. klotzchiana*, Basser (Voucher specimen 7393) collected in San Roberto, N.L. Both plants have the trivial name of "estafiate" and are used for stomach ailments.

Previous work. The *Artemisia* genus contains more than 300 species and geographical races many of which have been scrutinized by chemists [1]. Four santanolides, ludovicin-A, -B, -C [2] and ludolbin [3] has been isolated from *A. ludoviciana*, but only the essential oil of *A. klotzchiana* has been studied, GLC showing principally camphor and borneol.

Present work. The sesquiterpene lactones, deacetylmatricarin and achillin, originally obtained from other members of the same tribe the *Anthemideae*, have been found in both species and supports the concept of geographic races. The high yield of camphor and borneol from the essential oil of *A. ludoviciana* may have an important economical and chemotaxonomic value. The presence of 3,5-dihydroxy-6,7,8-trimethoxy flavone, an isomer of euparolin and eupatilin the cytotoxic flavonoids from *Eupatorium serratum* [5], also is of chemotaxonomic interest.

EXPERIMENTAL

Steam distillation. From 1 kg of the aerial part of fresh *A. ludoviciana*, 12 ml of yellowish oil were obtained, sp. gr.$^{24}_{4}$ 0·897; η_D^{25} 1·4764 $[\alpha]_D^{25}$ +2; GLC showed camphor, (52%) borneol (25%), phellandrene (4%), α-pinene (3%), plus 13 other minor constituents. As reported, the aerial part (1 kg) of *A. klotzchiana* afforded 8 ml of a bluish essential oil η_D^{25} 1·4665 $[\alpha]_D^{25}$, rich in camphor (35%) and borneol (40%).

Extraction of dried and milled plant material. Light petrol extraction of 498 g of *A. ludoviciana* afforded 1·22 g of achillin, mmp Co-TLC IR, NMR, [α] together with 0·84 g of camphor. *A. klotzchiana* (500 g) was extracted with $CHCl_3$ affording 15 g of resinous extract, which on Si gel chromatography gave 506 mg of deacetyl-matricarin mmp Co-TLC, IR, NMR, $[\alpha]_D^{25}$

and 57 mg of 3,5-dihydroxy-6,7,8-trimethoxyflavone, yellow plates mp 228–229° $C_{17}H_{14}O_7$ (M^+ 344) IR, 3400, 3030, 1650, 1570, 1550, 1490, 1450, 1400, 1350, 1310, 1290, 1250, 1200, 1140, 1100, 1090, 1030, 1010, 980, 950, 890, 850, 820, 780 cm^{-1}; chemical tests [6], MS, NMR ($CDCl_3$/TMS, in δ), 8·05 *d* (1H, 1Hz), 7·90 *d* (1H, 1Hz), 7·75 *d* (1H, 1Hz), 7·35 *d* (1H, 1Hz), 7·10 *s* (1H), 4·10 (*s*, 6H), 4·0 (*s*, 3H), UV λ_{max}^{MeOH}, 242 nm (ϵ 23243), 275 (22726), 340 (30991), bathochromic shifts of band I with $AlCl_3$, NaOMe, NaOAc as expected [7]. KOH fusion gave benzoic acid (TLC, PC and mmp).

Acknowledgments–We thank Dr. Jesús Romo, Instituto de Quimica U.N.A.M. for a sample of desacetylmatricarin; Prof. T. A. Geissman, UCLA for mass spectra, and CONACYT for financial support (Research Grant 015).

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Phytochemistry, 1975, Vol. 14, pp. 2512–2513. Pergamon Press. Printed in England.

PYRAZOLE IN *CITRULLIS VULGARIS* (CUCURBITACAE)*

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(*Received* 21 *April* 1975)

Key Word Index—*Citrullis vulgaris*; Cucurbitaceae; pyrazole; spectrophotometry.

L-β-Pyrazolyl-alanine and γ-L-glutamyl-β-pyrazolyl-L-alanine are found in seeds of many species of the Cucurbitaceae [1]. The former is synthesized by watermelon seedling extracts from pyrazole and *O*-acetylserine [2], while extracts of several other cucurbits were earlier reported to synthesize the compound from pyrazole and serine [3]; presumably, in these cases, the crude extracts could convert the amino acid to its *O*-acetyl derivative. A C_6H_6 extract of ground cucumber seeds heated with serine, pyridoxal phosphate and aluminum sulfate was shown to yield β-pyrazolyl-alanine [3], providing indirect evidence that pyrazole existed in the seed. However, the presence of free pyrazole was never confirmed nor its concentration determined at the time [3] for lack of a sensitive and specific assay.

In the presence of a mild oxidant, pyrazole forms a yellow complex with trisodium pentacyano-aminoferrate (TPF). This property has been applied to the quantitative analysis of pyrazole in microbial broths [4] and in blood [5]. We found that the TPF reagent could be used to demonstrate the presence of pyrazole in extracts of seed of *Citrullus vulgaris* (watermelon, *var* Coles Early and Early Canada). However, pyrazole was not detected in seed extracts of five varieties of squash (*Cucurbita melopepo*), 2 varieties of pumpkin (*Cucurbita pepo*), two varieties of cantaloupe (*Cucumis melo*), two varieties of cucumber (*Cucumis sativus*) and one variety of citron (*Citrullus vulgaris var citroides*).

The quantitative determination of pyrazole in watermelon seeds indicated a concentration of 410 μg/g in *var* Early Canada and 280 μg/g in *var* Coles Early. In 6-day old seedlings of *var* Early Canada, the concentration was 60 μg/g fr. wt in the root and 140 μg/g fr. wt in the hypocotyl.

Organic compounds containing the N–N bond have been reported in bacteria and fungi. Their presence in higher plants [1,6] is apparently rare, though this may reflect a lack of tests suitable for their detection. How plants synthesize or degrade the N–N bond remains a fascinating question for future study.

EXPERIMENTAL

Preparation of extracts. Seeds or other tissues were ground and extracted for 24 hr with 65% EtOH (3 to 5 ml/g) and extracts clarified by centrifugation at 3000 *g*.

* NRCC No. 14917.