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 ($CDCl_{3\,1}$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).

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PYRAZOLE IN *CITRULLIS VULGARIS* (CUCURBITACAE)*

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Key Word Index—*Citrullis vulgaris*; Cucurbitacae; 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*.

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* NRCC No. 14917.

Assay for pyrazole. From 0·2 to 0·5 ml extract was dil to 1·0 ml with 65% C_2H_5OH; 1·0 ml H_2O, 1·0 ml 0·2% trisodium pentacyanoaminoferrate, 0·1 ml 10% $NaNO_2$, and 0·1 ml conc HOAc were added with mixing. The absorptivity was determined at 458 nm.

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ESTERIFIED STEROL GLUCOSIDE AND METHYLELLAGIC ACIDS FROM *ALEURITES FORDII* FRUITS

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Key Word Index—*Aleurites fordii*; Euphorbiaceae; 6′-*O*-acylsterolglucoside; stigmasterol; campesterol; ellagic acid; 3,3′,4-tri-*O*-methylellagic acid; 3.3′-di-*O*-methylellagic acid; methyl gallate.

Plant. Aleurites fordii Hemsl. *Source.* Okayama University campus and Handayama Botanical Garden. *Uses.* Tung oil has been used in paints, varnishes, etc. *Previous work.* On the toxic principle of fruits [1], on the amino acids of seeds [2], and on the sterols of tung oil [3,4].

Present work. Fresh fruits were separated into seeds and residual part (outer part), and chemical constituents of each part were examined.

Constituents of seeds. Powdered seeds were extracted with petrol. After removal of solvent, the residual oil was shaken vigorously with MeOH and decanted. This procedure was repeated several times. The combined MeOH solutions were evaporated and chromatographed over Si gel. Elution with $CHCl_3$ gave a crystalline *sterol*, mp 136° (EtOH), $[\alpha]_D$ $-40°$ ($CHCl_3$). GLC on a column of 2% OV-17 on Chromosorb W showed it to be a mixture of sitosterol (84%), stigmasterol (11) and campesterol (5). The identities with authentic samples were confirmed on GC–MS. Elution with $CHCl_3$–Me_2CO (9:1) gave another substance positive to Liebermann–Burchard reaction, which was further purified by PLC on Si gel developing with $CHCl_3$–Me_2CO (3:1) to afford an *esterified sterol glucoside* (ESG) as a colourless syrup. It was homogeneous on TLC ($CHCl_3$–Me_2CO 3:1, R_f 0·11; C_6H_6–Me_2CO 3:2, R_f 0·33). $\nu_{max}^{CHCl_3}$: 3550, 3400, 1725, 1630(*sh*) cm^{-1}. Treatment with Ac_2O-pyridine gave triacetate, mp 126–128° (MeOH), $[\alpha]_D$ $-20°$ ($CHCl_3$), analysed for $C_{57}H_{96}O_{10}$, ν_{max}^{KBr}: 1747, 1635, 1250 cm^{-1}. NMR (90 MHz, $CDCl_3$) δ 1·98, 2·00, 2·02 (3 OAc). ESG and its acetate were methanolysed to give mixed sterol glucosides, mp 283° (decomp.), and fatty acid methyl esters mixture which was shown by GLC (1% OV-1) to be composed by methyl palmitate (58%), methyl linolate (27), and methyl stearate (15). Acid hydrolysis of the sterol glucosides mixture yielded glucose (PC, TMSi–GLC) and mixed sterols, mp 136°, which was shown by GLC and GC–MS to comprise sitosterol (78%), stigmasterol (18) and campesterol (4). The location of the ester group in ESG was shown to be mainly at C-6′ by NMR (90 MHz, $CDCl_3$) spectrum; methylene signal of C-6′ overlapped with the anomeric proton signal at δ 4·31, and no downfield shift of C-6′ protons was shown by the acetate (δ 4·13, *m*). The major component of ESG is, therefore, 6′-acylglucoside of sitosterol. Elution of the column with $CHCl_3$–Me_2CO (7:3) gave mixed *sterol glucoside*, mp 293° (decomp.) (pyridine–EtOH), $[\alpha]_D$ $-42°$ (pyridine), analysed for $C_{35}H_{60}O_6$; tetraacetate, mp 164–165° (MeOH), $[\alpha]_D$ $-24°$ ($CHCl_3$), analysed for $C_{43}H_{68}O_{10}$. Acid hydrolysis yielded glucose (PC, TMSi–GLC) and sterol mixture, mp 138° (EtOH), which is shown by GC–MS to be composed by sitosterol (85%), stigmasterol (12) and campesterol (3).