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CUCURBITACINS AND AROMATIC COMPOUNDS FROM *CRINODENDRON HOOKERIANUM**

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Abstract—Cucurbitacins-D, -F, -H, and the new member 23,24-dihydrocucurbitacin F have been isolated from *Crinodendron hookeranium*. This is the first report of cucurbitacins in the Elaeocarpaceae. Other components isolated included gallic and ellagic acids, methyl 3-*O*-methylgallate and an ellagitannin.

INTRODUCTION

ORIGINALLY isolated as the bitter principles of the Cucurbitaceae,[1] the cucurbitacins have more recently been reported in the Cruciferae,[2] Scrophulariaceae[3] and Begoniaceae.[4] The present investigation adds the Elaeocarpaceae to this number. An authoritative review on the cucurbitacins has recently been published,[5] which includes details and evidence for the structures of these compounds. One final point of contention in the structural assignments is the configuration of the hydroxyl group about position-2 in the appropriate members. This has been assigned on the basis of extensive CD evidence as 2α,[5] but a recent X-ray analysis upon datiscoside, a glycoside derivative of curcurbitacin-D, after derivatization to its iodobenzoate ester, showed the 2β-orientation.[6]

The tumour-inhibiting properties of the cucurbitacins are well known.[4,7] In the present instance, extracts derived from the aqueous alcoholic plant extract showed significant inhibition, *in vivo*, against P-388 lymphocytic leukaemia (PS) in mice.‡ However, none of

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‡ Cytotoxicity (KB) and *in vivo* (PS) tests were carried out at the National Cancer Institute, N.I.H. For Procedures see (1962) *Cancer Chemother. Rep.* **25**, 1.

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[6] KUPCHAN, S. M., SIGEL, C. W., GUTTMAN, L. J., RESTIVO, R. J. and BRYAN, R. F. (1972) *J. Am. Chem. Soc.* **94**, 1353.
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the pure compounds isolated so far is responsible for this activity, although the cucurbitacins did show *in vitro* activity against cells derived from human carcinoma of the nasopharynx (KB). It is anticipated that cucurbitacin glycosides are responsible for the observed PS activity.[6,8]

RESULTS

A chloroform extract of the leaves and stems of *Crinodendron hookerianum* Gay (see Experimental) afforded three components. The major, least polar compound was methyl 3-*O*-methylgallate, confirmed by its characterization as the diacetate and the trimethyl ether. The remaining compounds in this extract were cucurbitacins. Of these the major compound showed a fragmentation pattern consistent with cucurbitacin-D (I) (see Table 1),[9] which was confirmed by direct comparison with an authentic sample and by comparison of its derived diacetate. Addition of diazomethane across the 23-en-22-one function afforded a pyrazoline derivative, presumably with the side chain as in (II).

TABLE 1. MS FRAGMENTATION PATTERNS OF CUCURBITACINS*†

D (I): 516 (M^+), 498, 455, 403, 385, 369, 111, 96
D-diacetate (I; R = Ac, R′ = H): 582 (M^+–H_2O), 522, 487, 462, 427, 410, 367, 111, 96
F (VI): 500 (M^+), 482, 457, 405, 403, 387, 219, 171, 135, 111, 96.
Dihydro-F (V): 502 (M^+), 484, 469, 459, 451, 405, 387, 369, 166, 142, 113, 97, 95
H (VII): 516 (M^+), 498, 484, 455, 418, 404, 386, 371, 368, 353, 191, 177, 157, 135, 132, 93, 91

* Carried out at 70 eV; most prominent peaks quoted, besides M^+.
† See Refs. 9 and 10 for previous studies on cucurbitacins.

A thorough examination of the chloroform extract by multiple-elution TLC and comparison with authentic samples showed that cucurbitacins-A, -B, -C and -E were absent and the same was true for the light petrol. and benzene extracts. That cucurbitacin-B is not present in the plant is of significance, since in the Cucurbitaceae[1] compound-D has always been reported in conjunction with B. However, Curtis and Meade[2] have found that compound-D is found in the absence of B in *Iberis gilraltarea*, a member of the Cruciferae.

The third, more polar and minor, fraction of the chloroform extract also contained triterpenes and showed peaks at *m/e* 520, 518, 502 and 500, indicating a mixture. Since the UV absorption at λ_{max} 229 nm was less than that for cucurbitacins containing the 23-en-22-one system, a mixture of saturated and unsaturated cucurbitacins was suggested. Since the cucurbitacins O (III) and P (IV) are also difficult to separate and have similar spectral properties[10] a comparison with these compounds was made. Although these triterpenes and the minor chloroform extract had the same R_f in several solvents, they could be differentiated both by the differences in their response to spray reagents, such as 6 N H_2SO_4 and in their MS fragmentation patterns. Finally, multiple elution TLC eventually allowed the extract to be separated into its two components. The more polar compound had MS fragmentation peaks at 520, 459 and 405 (see Table 1) and comparison with a sample of

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[9] AUDIER, H. E. and DAS, B. C. (1966) *Tetrahedron Letters* 2205.
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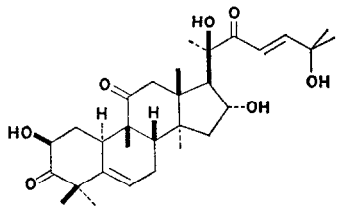
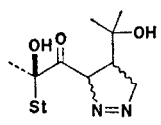
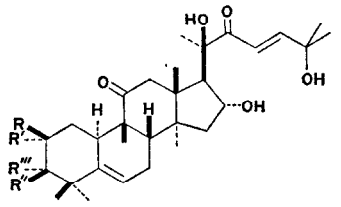
23,24-dihydrocucurbitacin-F (V), synthesized[11] from authentic cucurbitacin-F (VI) showed them to be identical. The less polar material was, in fact, further contaminated by small quantities of another compound as evinced by peaks at *m/e* 498, 455 and 404 and 403 in its MS. Small peaks at these values were also noticed in the mixture before multiple elution TLC. Crystallization of this fraction afforded a small quantity of the major component, identified as cucurbitacin-F. The very minor component could not be isolated from the mother liquors. This latter substance was isolated, however, after catalytic reduction of the unseparated dihydro-F and F mixture. Multiple elution TLC separated out all the dihydro-F component and afforded a small quantity of the minor triterpene, which showed the 'contaminant' MS fragmentation pattern only. This was shown, by spectral comparison, to be cucurbitacin-H (VII).[12]



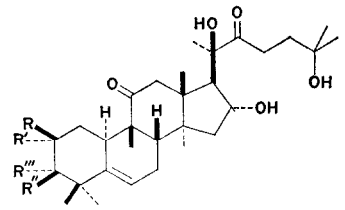
(I)



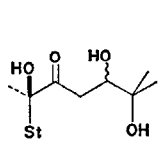
(II)



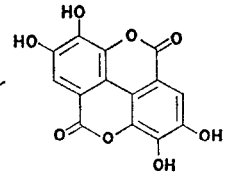
(III) R = R″= OH; R′= R‴= H
(VI) R = R‴= OH; R′= R″= H



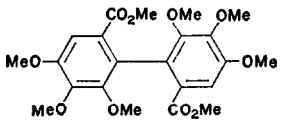
(IV) R = R″= OH; R′= R‴= H
(V) R = R‴= OH, R′= R″= H



(VII) St as in (I)



(VIII)



(IX)

A search for the less polar-24-epimer, cucurbitacin-G (VII, isomer at 24)[1] resulted only in the isolation of unidentified compounds in an impure state, thought to be due to ring A oxidation of the cucurbitacin-F and its dihydro derivative.

Other constituents isolated from the ethyl acetate from this plant were all aromatic. Silica gel and cellulose chromatography afforded 3-*O*-methylgallate, gallic acid,[13] ellagic acid (VIII)—identified by conversion into its acetate and tetra-*O*-methyl ether[14]—and an ellagitannin. Acid hydrolysis of the latter indicated that it contained glucose and gallic, ellagic and 3,4-di-*O*-methylgallic acids. Methylation of the ellagitannin under basic conditions also afforded a small quantity of an octamethylated hexahydroxydiphenic acid (IX).[15]

The PS assays on the isolated compounds were disappointing, showing insignificant activities; cucurbitacin-D was toxic at the tested dosages (6·6 mg/kg animal).

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EXPERIMENTAL*

Extraction. The dried, powdered leaves and stems of *Crinodendron hookerianum* (8·4 kg) were extracted with 50% alcohol–H_2O at 50° and the extract fractionated by sequential extraction with light petrol, C_6H_6, $CHCl_3$, EtOAc, BuOH and MeCOEt. Based on tumour-inhibiting assays, only the $CHCl_3$ and EtOAc extracts were examined.

$CHCl_3$ *extract.* (a) PLC, in EtOAc, of the $CHCl_3$ extract (1·42 g of 26 g) gave methyl 3-*O*-methylgallate (574 mg, 0·1%), m.p. 111–112·5° (Et_2O–hexane) (lit.[16] m.p. 116°), ν_{max}^{Nujol} 3440, 3390, 1685, 1615 cm^{-1}, λ_{max} 276 and 219 (ϵ 12000 and 28000), M^+ 198. (Found: C, 54·81; H, 5·26 Calc. for $C_9H_{10}O_5$: C, 54·60; H, 5·09%.) The diacetate, obtained with pyridine–Ac_2O, had m p. 96–97° (lit [16] m.p. 99°), whilst its dimethyl-ether, obtained by treatment with CH_2N_2 had m p. 80–83° (lit.[17] m.p. 82 5°). Hydrolysis of the ester afforded 3,4,5-trimethylgallic acid as needles, m.p. 168–169° (lit.[16] m p. 167°) (b) The next compound (more polar) was cucurbitacin-D (134 mg; 0·03%), m p 144–145° (EtOAc), $[\alpha]_D^{25}$ +42° (*c* 0·98, $CHCl_3$), [18] λ_{max} 229 nm (ϵ 10400), ν_{max}^{Nujol} 3418, 1717, 1690 and 1630 cm^{-1}. A m.m p. with an authentic sample was undepressed and TLC comparison was identical Acetylation with Ac_2O–pyridine overnight at room temp afforded the diacetate, again identical to an authentic sample, and with *m/e* 582 (M^+–H_2O), 522, 487, 462, 427, 410, 367, 111, 96. Prolonged exposure to CH_2N_2 in Et_2O converted the cucurbitacin-D into a monopyrazoline adduct, m.p. 160° (MeOH), ν_{max}^{Nujol} 3400, 1712, 1695, 1625 and 1123 cm^{-1}, *m/e* 512 (M^+–N_2–H_2O), 494, 403, 402, 384, 369, 142, 125, 113 and 104. (c) The most polar fraction from the PLC separation of this extract gave crystals (40 mg, 0 01%), m p 172–172·5° (EtOH–C_6H_6), λ_{max}^{EtOH} 229 nm (ϵ 6700), *m/e* 520, 518, 516, 502, 500, 498, 484, 482, etc (Found: 484·3197, 457 2955. Calc. for $C_{30}H_{44}O_5$: 484·3187, calc. for $C_{28}H_{41}O_5$: 457·2954.) An authentic mixture of curcurbitacins-O and -P showed an almost identical IR spectrum and a similar MS fragmentation pattern. However, multiple elution PLC (3:7, C_6H_6–EtOAc, SiO_2 G, 5 elutions) enabled the mixture to be separated into two components; cucurbitacins-O and -P were separated under similar conditions to give components of similar R_fs but these latter triterpenes gave a different colour response to 6 N H_2SO_4 (grey–brown; former gave red–purple colours). The more polar component of the mixture was re-chromatographed to give 23,24-dihydrocucurbitacin-F as an amorphous solid, m.p. 128–135°, $[\alpha]_D^{25}$ +50° (*c* 0·47, EtOH), λ_{max}^{EtOH} end absorption only, ν_{max}^{Nujol} 3440, 1691, 1684, 1220 (weak), 1215, 1055, 1030 cm^{-1}, *m/e* 520, 502, 484, 469, 459, 451, 405, 387, 369, 166, 142, 113, 97 and 95 (Found: 484·3173. Calc. for $C_{30}H_{44}O_5$· 484 3189.) M m.p with an authentic sample[11] (m p 135–140°) was undepressed; its TLC and spectral properties were also identical. Hydrogenation of the crude polar fraction over 10% Pd–C at room temp and pressure increased the yield of dihydrocucurbitacin-F. The less polar part (*ca.* 60%) of this cucurbitacin fraction contained cucurbitacin-F and -H. Repeated crystallization (EtOH–EtOAc) gave almost pure cucurbitacin-F, m p 174–178°, $[\alpha]_D^{25}$ +32° (*c* 0·67, EtOH), λ_{max}^{EtOH} 231 nm (ϵ 8900), ν_{max}^{Nujol} 3410, 1687, 1630, 1287, 1215, 1059, 1030 cm^{-1}, identical TLC and MS fragmentation properties to an authentic sample. Chromatography of the hydrogenated crude fraction (PLC, multiple elutions) afforded a small sample of cucurbitacin-H,[1] m.p. 127–129° (from EtOH), ν_{max}^{Nujol} 3400, 1693, 1270, 1210, 1058 and 1030 cm^{-1}. (Found: C, 64·65; H, 8·52 Calc. for $C_{30}H_{46}O_8.H_2O$: C, 65·19, H, 8·75) Examination of the crude extract for cucurbitacin-G was negative.

EtOAc *extract.* Column chromatography (SiO_2) of a sample of this extract gave methyl 3-*O*-methylgallate, identical to the material obtained previously, and gallic acid, m.p. 224–226° (aq EtOH) (lit.[13] m p 225–250°), which gave a triacetate m p. 159–162° (lit.[13] m.p. 170°). Chromatography on cellulose powder (80 g) of the extract (2·1 g) gave an ellagitannin (EtOAc) (400 mg) as a pale yellow solid. Purification on Whatman No. 3 paper (descending chromatography, 1:6 HOAc–H_2O) gave pale yellow granules (160 mg), m p. 235–240° $[\alpha]_D^{25}$ −119° (*c* 1·4, EtOH), ν_{max}^{Nujol} 3400, 2720, 1730, 1700, 1610, 1510, 1200, 1088, 1035, 968, 765 cm^{-1} λ_{max}^{EtOH} 220, 279 nm; it gave a dark green colour with $FeCl_3$. Acetylation with pyridine–Ac_2O gave an acetate which still showed hydroxyl absorption bands in its IR spectrum. Methylation (CH_2N_2 Et_2O–MeOH) afforded a crude yellow compound, which was purified by PLC to give a mixture, identified mainly by MS as octamethylhexahydroxydiphenic acid (IX) (*m/e* 450), tetra-*O*-methylellagic acid (*m/e* 358) and methyl tri-*O*-methylgallate (*m/e* 226). Acid hydrolysis of the ellagitannin (63 mg) in refluxing ethanolic 1 N HCl for 10 hr gave a beige precipitate (20 mg), identified as ellagic acid,[14] m.p 360° (dec), ν_{max}^{Nujol} 3560, 3300–3100, 1700–1690, 1618, 1583, 1115, 1060 cm^{-1} λ_{max}^{EtOH} 254 and 358 nm *m/e* 302 M^+) It gave a tetra-acetate, m.p. 310–316° and a tetramethyl ether, m p 335–339° (lit.[14] m.p. 341°) An examination of the acid layer showed glucose as the only sugar present. EtOAc extraction of this acid layer gave gallic acid and a less polar aromatic

* For details of instrumentation see SILVA, M , CRUZ, M. A. and SAMMES, P. G. (1971) *Phytochemistry* **10**, 3255. Microanalyses were carried out by Mr K. Jones, Chemistry Department, Imperial College, London PLC signifies preparative TLC.

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[17] *Dictionary of Organic Compounds* (1953 Edn), Vol II, p 583, Eyre & Spottiswood, London.
[18] WRZECIONO, U. (1969) *Wiad Chem* **23**, 415.

acid, provisionally assigned as 3,4-di-*O*-methylgallic acid (*m/e* 198, 183, 181, 169, 155, 153, 140, 137, 127, 109 and 93).

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