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A CUCURBITACIN GLYCOSIDE FROM *PICRORHIZA KURROOA*

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Key Word Index—*Picrorhiza kurrooa*; Scrophulariaceae; kutki; kutkin; isolation; structure determination; cucurbitacin glycoside.

Abstract—A novel cucurbitacin glycoside bitter principle has been isolated from root extracts of *Picrorhiza kurrooa* and shown to be 25-acetoxy-2-β-glucosyloxy-3,16,20-trihydroxy-9-methyl-19-norlanosta-5,23-diene-22-one. Two previously described catalpol derivatives were also isolated; neither was as bitter as the cucurbitacin glycoside.

INTRODUCTION

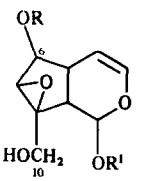
The bitterness of extracts of the rhizome and roots of *Picrorhiza kurrooa* Royle and Benth. is attributed to the presence of picroside-I (**1**, 6′-cinnamoylcatalpol) [1, 2]. Other catalpols [3, 4] have been isolated from the plant but none are reported to be bitter. Kutkin, an earlier described bitter principle [5], is now recognized to be a stable mixed crystal of picroside-I and a second catalpol. Although the latter was identified as 10-vanilloylcatalpol [2], later work [3] would suggest that it is 6-vanilloylcatalpol (**2**). The observation that crystallisation of crude kutkin increased the catalpol content but decreased the bitterness prompted a search for other bitter principles in the extract.

RESULTS AND DISCUSSION

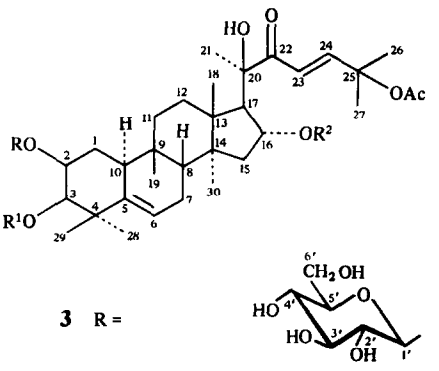
TLC established that crystallization of crude kutkin reduced substantially the concentration of a minor component which migrated faster than **1**. A compound showing a similar chromatographic behaviour has been noted in kutkin previously [2, 3] though not characterized. The minor component was isolated by column chromatography and found to be appreciably more bitter than picroside-I. On the basis of spectroscopic studies the new bitter principle has been identified as the cucurbitacin glycoside **3**. In contrast to other cucurbitacins there is no ketone function at C-11.

Treatment of **3** with an enzyme possessing β-glucosidase activity readily gave the aglycone **4** for which the molecular weight was shown to be 546 by CIMS. The molecular formula $C_{32}H_{50}O_7$ was deduced from mass measurement of $[M-60]^+$, the highest observable ion in the EIMS. Absence of a molecular ion due to facile loss of acetic acid is a known feature of cucurbitacin mass spectra [6] which also frequently show a base peak at m/z 96 as observed in the case of **4**.

The ^{1}H NMR spectrum of the aglycone showed similarities to literature data for cucurbitacins [7]. Signals attributable to an acetyl group (at δ2.01) and eight other methyls were present. Three one proton signals occurred in the olefinic region (δ7.04, 6.48 and 5.65). The two lower field signals comprised an AB system with a coupling constant (15.5 Hz) characteristic of a *trans*-double bond and were assigned to the α,β-unsaturated ketone function known to be present (ν1690 cm^{-1} and λ_{max} 230 nm, log ε4.07). The conclusion was that the aglycone possessed the normal [7, 8] cucurbitacin side chain with an acetate function at C-25. A further interesting feature of the ^{1}H NMR spectrum of **4** was the presence of signals at δ4.33, 4.0 and 3.47 which were attributable to protons attached to hydroxylated carbons. The signals at δ4.0 (*br d*, J = 11.5, 4 and 2 Hz) and δ3.47 (*d*, J = 2 Hz) were



Picroside - I (**1**) R = H, R^1 = 6 - cinnamoyl - β - D - glucopyranosyl
Picroside - II (**2**) R = vanilloyl, R^1 = β - D - glucopyranosyl



3 R = , R^1 = R^2 = H

4 R = R^1 = R^2 = H
8 R = R^1 = R^2 = Ac
9 R = R^2 = Ac, R^1 = H
10 R = tetra - *O* - acetyl - β - D - glucopyranosyl, R^1 = R^2 = Ac
11 R = tetra - *O* - acetyl - β - D - glucopyranosyl, R^1 = H, R^2 = Ac

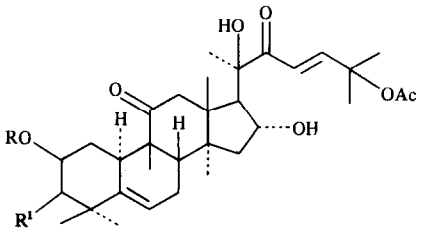
mutually coupled and were assigned to H-2 and H-3, respectively. The 2β,3β-diol configuration has been firmly established in cucurbitacins O, P and S [9]. The location of the remaining hydroxyl at C-16 in the D-ring, a standard cucurbitacin position [6, 7], was consistent with the structure of the signal at δ4.33 (*t*, *J* = 7.5 Hz). A very similar signal was present in the spectrum of cucurbitacin E (**5**). Further evidence for the proposed location of the hydroxyl functions in **4** was obtained by reaction with methyl boronic acid. The mass spectrum of the product showed molecular ions at *m/z* 594, 593 and 592 indicative of a di-adduct containing two cyclic boronate systems (at C-2, 3 and C-16, 20). The fragmentation pattern having the base peak at *m/z* 96 was consistent with this structure.

Increasing use is being made of ^{13}C NMR spectroscopy in the structural elucidation of cucurbitacins [10–12]. The carbon spectra of **3** and **4** and published data for cucurbitacin E (**5**) [10], arvenin I (**6**) [10] and cucurbitacin II glycoside (**7**) [11] were compared (Table 1). Only two carbonyl carbon signals were present in the spectra of both **3** and **4** and as these occurred at chemical shifts appropriate for the C-22 α,β-unsaturated ketone and an acetate carbonyl there could be no ketone function at C-11. One of the methylene carbon signals was therefore due to C-11. The signals in the C–O region could be assigned to C-2, C-3, C-16, C-20 and C-25. Chemical shift differences were apparent for the remaining D-ring carbon atoms and for the C-18 and C-30 methyl groups which in other cucurbitacins characteristically occur very close to 20 ppm [12]. Comparison of the spectra for **3**, **6** and **7** indicated that the same sugar residue, namely β-D-glucopyranosyl, was present at the same location in all three compounds. The C-2 chemical shift difference between **3** and **4** was however not so marked as in the case of **7** and the corresponding aglycone [11]. The location and nature of the sugar residue was confirmed by ^1H NMR analysis of the acetylated derivatives of **3** and **4**. Acetylation of **4** gave 2,3,16,25-tetraacetate **8** together with 2,16,25-triacetate **9**. These products were readily identified by ^1H NMR spectroscopy because of the changes in chemical shift of the characteristic signals due to H-2, H-3 and H-16 (see Table 2).

Acetylation of **3** yielded heptaacetate **10** and hexaacetate **11**. Here as with **4** the reaction of the hydroxyl at C-3 was incomplete. In accord with previous experience [7] the C-20 hydroxyl groups of **3** and **4** failed to acetylate. Neither **10** nor **11** had an acetate function at C-2 (see Table 2) and so the location of the sugar residue at this position was confirmed.

The magnitude of the coupling constants observed for the sugar ring protons in the NMR spectra of both **10** and **11** showed that all were axial and the chemical shifts of the signals were very close to those reported for methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside [13].

The presence of a small amount of a dihydro impurity in **4** was indicated by mass spectrometry and this probably accounted for the melting point range (174–181°; aqueous methanol). Several cucurbitacins (e.g. **7**) lack the 23,24-double bond and it is likely that the impurities in **4** and hence also in **3** were 23,24-dihydro derivatives.



Cucurbitacin E (**5**) R = H, R¹ = ═O, 1,2-dehydro
Arvenin I (**6**) R = β-D-glucopyranosyl, R¹ = ═O
Cucurbitacin II glycoside (**7**) R = β-D-glucopyranosyl
R¹ = –OH, ---H, 23,24-dihydro

Table 1. Carbon shifts of cucurbitacins 3–7 (δ values, C_5D_5N containing TMS standard)

Carbon	3	4	5	6	7
C-1	34.7*	32.2*	115.8	34.4	33.2
2	76.7†	68.9	147.2	78.2	83.2
3	75.7†	79.8	198.7	211.3	80.6
4	41.6	42.0	48.6	48.5	42.5
5	141.6	141.9	137.9	140.8	141.7
6	119.9	119.9	120.6	120.4	119.0
7	25.3	25.3	24.0	24.2	25.2
8	34.7*	34.8	35.3	35.0	34.3
9	43.0	43.0	48.6	48.9	48.6
10	37.6	37.4	42.3	42.9	43.1
11	30.8*	30.9*	213.5	212.7	212.9
12	32.0*	30.9*	49.4*	49.1*	49.3*
13	46.7	46.8‡	49.4‡	51.0‡	48.8‡
14	46.7	49.0‡	51.0‡	51.5‡	51.0‡
15	48.9	49.0	46.6*	46.1*	46.3*
16	71.4	71.2	70.9	70.8	70.4
17	60.1	60.2	59.7	59.6	58.9
20	80.2	80.1	79.7	79.8	81.6
22	204.4	204.1	204.1	204.0	214.9
23	122.8	122.8	122.6	122.5	32.1
24	149.7	150.0	150.1	150.0	35.4
25	80.2	80.1	79.7	79.8	80.0
OCOMe	169.7	169.7	169.7	169.7	170.1
	18.5	18.5	18.5	18.9	19.0
	18.6	18.6	20.2	19.9	20.2
	21.8	21.8	20.5	20.4	20.2
	24.6	24.6	20.7	20.4	22.2
Me	26.2	26.2	21.7	21.8	22.2
	26.2	26.2	25.4	25.3	24.1
	26.7	26.7	26.3	26.3	25.4
	27.2	27.2	26.6	26.6	26.0
	28.0	28.2	28.1	28.7	26.1
1′	101.3			104.0	106.3
2′	75.7			75.6	75.8
3′	78.5			78.2	78.4
4′	71.8			71.4	71.5
5′	78.5			78.2	78.3
6′	62.8			62.6	62.6

*†‡Assignments may be reversed in each vertical column.

Table 2. ^1H NMR chemical shifts (δ) of relevant protons in compounds **4** and **8–11**

	4	8	9	10	11
H-2	4.0	5.24	5.22	4.06	4.07
H-3	3.47	5.01	3.55	5.0	3.50
H-16	4.33	5.18	5.17	5.19	5.18

Direct extraction of kutkin from the dried roots of four different varieties of *P. kurrooa* revealed considerable variation in the content of **3**, which reached a maximum of ~ 15% of the total kutkin extracted in the case of 'small black' variety of the species.

Cucurbitacin glycoside **3** had a bitterness threshold of below 1 ppm compared with 5 ppm for picroside-I (**1**). Compound **2** was without bitterness.

EXPERIMENTAL

The dried root material (10 g) was defatted (petrol) then extracted with MeOH–$CHCl_3$ (1:4). The crude extract (1.45 g) was separated by CC (silica gel; MeOH–$CHCl_3$, 1:4) affording 140 mg 25-acetoxy-2-β-D-glucosyloxy-3,16,20-trihydroxy-9-methyl-19-norlanosta-5,23-diene-22-one (**3**) and 360 mg **2**. Alternatively crude kutkin (1.36 g) was likewise separated affording 11 mg **3**, 350 mg **1** and 450 mg **2** together with 55 mg of material eluting between **1** and **2** and having a ^{1}H NMR spectrum consistent with that of a mixture containing picroside-III [4] and a cucurbitacin or steroid. The known compounds **1** and **2** were identified by comparing their ^{1}H and ^{13}C NMR spectra with those of authentic materials. Compound **3**, mp 127–137°; ^{1}H NMR (220 MHz, CD_3OD): δ1.02 (*s*, 3H), 1.11 (*s*, 6H), 1.19 (*s*, 3H), 1.27 (*s*, 3H), 1.48 (*s*, 3H), 1.62 (*s*, 3H), 1.64 (*s*, 3H), 1.85 (*cm*), 2.09 (*s*, 3H), 2.5 (*cm*, 3H), 3.3–5.2 (*cm*), 5.7 (*br s*, 1H, H-6), 6.79 (*d*, 1H, H-23, $J_{23,24}$ = 17.5 Hz), 7.08 (*d*, 1H, H-24); IR ν_{max}^{nujol} cm^{-1}: 3380, 1720, 1690, 1265; UV λ_{max}^{MeOH} (log ε): 228 (3.99).

25-Acetoxy-2,3,16,20-tetrahydroxy-9-methyl-19-norlanosta-5,23-diene-22-one (**4**). A soln of cucurbitacin glycoside **3** (84 mg) in EtOH (0.4 ml) was added to a soln of naringinase (200 mg in 10 ml H_2O at pH 5.6). After 23 hr at 37° the mixture was subjected to conventional extraction with $CHCl_3$ to yield crude aglycone (59 mg). After purification by prep. TLC (silica gel; 1 mm; MeOH–$CHCl_3$, 2:98), compound **4** was obtained as a colourless glass (38 mg). Mp 174–181° (aq. MeOH), ^{1}H NMR (250 MHz, $CDCl_3$): δ0.97 (*s*, 3H), 1.03 (*s*, 3H), 1.08 (*s*, 6H), 1.20 (*s*, 3H), 1.25 (*t*, 2H, *J* = 6.6 Hz), 1.44 (*s*, 3H), 1.56 (*s*, 6H), 1.6–1.84 (*m*, 8H), 2.01 (*s*, 3H), 2.28 (*d*, 1H, *J* = 7.2 Hz, H-17), 2.32 (*m*, 2H), 2.65 (*br*, 4H, OH), 3.47 (*d*, 1H, *J* = 2 Hz, H-3), 4.0 (*br d*, 1H, *J* = 11.5 Hz, H-2), 4.33 (*t*, *J* = 7.5 Hz, H-16), 5.65 (*br s*, 1H, *J* = 5.5 Hz, H-6), 6.48 (*d*, 1H, $J_{23,24}$ = 15.5 Hz, H-23), 7.04 (*d*, 1H, H-24); IR ν_{max}^{film} cm^{-1}: 3450, 1735, 1720, 1690, 1632, 1250. EIMS (probe) 70 eV, *m/z* (rel. int.): 486 [M − HOAc]$^+$ (3.5), 96 (100). Mass measurements 486.3394 ($C_{28}H_{40}O_5$ = 486.3345) 96.05716 (C_6H_8O = 96.0575).

Acetylation of **3**. Cucurbitacin glycoside **3** (24 mg) was subjected to conventional acetylation. The crude product was separated by preparative TLC (silica gel; MeOH–$CHCl_3$, 3:97) to yield 20 mg **10** and 12 mg **11**.

Compound **10**. ^{1}H NMR (250 MHz, $CDCl_3$): δ0.94 (*s*, 3H), 1.04 (*s*, 3H), 1.08 (*s*, 3H), 1.15 (*s*, 3H), 1.26 (*s*, 6H), 1.43 (*s*, 3H), 1.58 (*s*, 6H), 1.84 (*s*, 3H), 2.0 (*s*, 3H), 2.0 (*s*, 3H), 2.02 (*s*, 3H), 2.03 (*s*, 3H), 2.06 (*s*, 3H), 2.12 (*s*, 3H), 2.35 (*br m*, 2H), 2.47 (*d*, 1H, *J* = 7.3 Hz), 3.74 (*dt*, 1H, *J* = 10, 3 Hz, H-5'), 4.06 (*br d*, 1H, *J* = 11.5 Hz, H-2), 4.27 (*m*, 2H, 2 × H-6'), 4.7 (*d*, 1H, $J_{1',2'}$ = 8 Hz, H-1'), 4.9 (*dd*, 1H, *J* = 10, 8 Hz, H-2'), 5.0 (*br s*, 1H, H-3), 5.09 (*t*, 1H, *J* = 9.5 Hz), 5.19 (*t*, 1H, *J* = 7.2 Hz, H-16), 5.22 (*t*, 1H, *J* = 9.5 Hz), 5.53 (*br d*, 1H, *J* = 5 Hz, H-6), 6.41 (*d*, 1H, $J_{23,24}$ = 15.5 Hz, H-23), 7.14 (*d*, 1H, H-24).

Compound **11**. ^{1}H NMR (250 MHz, $CDCl_3$): δ0.94 (*s*, 3H), 1.01 (*s*, 3H), 1.03 (*s*, 3H), 1.12 (*s*, 3H), 1.20 (*s*, 3H), 1.41 (*s*, 3H), 1.57 (*s*, 6H), 1.83 (*s*, 3H), 2.02 (*s*, 3H), 2.04 (*s*, 3H), 2.07 (*s*, 3H), 2.11 (*s*, 3H), 2.34 (*br d*, 2H), 2.48 (*d*, 1H, *J* = 7.5 Hz), 3.50 (*br s*, 1H, H-3), 3.74 (*dq*, 1H, *J* = 9.5, 4.5, 2.5 Hz, H-5'), 4.07 (*br d*, 1H, *J* = 11 Hz, H-2), 4.20 (*dd*, 1H, *J* = 12.5, 2.5 Hz), 4.28 (*dd*, 1H, *J* = 12.5, 4.5 Hz), 4.71 (*d*, 1H, *J* = 8 Hz, H-1'), 4.99 (*dd*, 1H, *J* = 9.5, 8 Hz, H-2'), 5.13 (*t*, 1H, *J* = 9.5 Hz), 5.18 (*t*, 1H, *J* = 7.5 Hz, H-16), 5.24 (*t*, 1H, *J* = 9.5 Hz), 5.62 (*br d*, 1H, *J* = 5 Hz, H-6), 6.43 (*d*, 1H, $J_{23,24}$ = 15.5 Hz, H-23), 7.14 (*d*, 1H, H-24).

Acetylation of **4**. Aglycone **4** (17 mg) was acetylated and the crude product separated by TLC (silica gel, $CHCl_3$) to yield 10 mg **8** and 5 mg **9**.

Compound **8**. ^{1}H NMR (250 MHz, $CDCl_3$): δ0.96 (*s*, 3H), 1.03 (*s*, 3H), 1.04 (*s*, 3H), 1.14 (*s*, 6H), 1.41 (*s*, 3H), 1.58 (*s*, 3H), 1.585 (*s*, 3H), 1.83 (*s*, 3H), 2.00 (*s*, 3H), 2.03 (*s*, 3H), 2.07 (*s*, 3H), 2.42 (*br m*, 2H), 2.47 (*d*, 1H, *J* = 7.3 Hz), 5.01 (*d*, $J_{2,3}$ = 2.2 Hz, H-3), 5.18 (*t*, 1H, *J* = 7.5 Hz, H-16), 5.2 (*br d*, *J* = 11.6 Hz, H-2), 5.56 (*d*, *J* = 5.0 Hz, H-6), 6.42 (*d*, $J_{23,24}$ = 15.6 Hz, H-23), 7.14 (*d*, H-24); CIMS(NH_3) *m/z*: 690.

Compound **9**. ^{1}H NMR (250 MHz, $CDCl_3$): δ0.95 (*s*, 3H), 1.03 (*s*, 3H), 1.08 (*s*, 3H), 1.12 (*s*, 3H), 1.20 (*s*, 3H), 1.42 (*s*, 3H), 1.58 (*s*, 3H), 1.584 (*s*, 3H), 1.83 (*s*, 3H), 2.04 (*s*, 3H), 2.11 (*s*, 3H), 2.44 (*br d*, 2H), 2.48 (*d*, 1H, *J* = 7.35 Hz), 3.55 (*br s*, 1H, H-3), 5.17 (*t*, 1H, *J* = 7.7 Hz, H-16), 5.22 (*br d*, 1H, H-2), 5.65 (*d*, 1H, *J* = 6.5 Hz, H-6), 6.42 (*d*, 1H, $J_{23,24}$ = 15.4 Hz, H-23), 7.14 (*d*, 1H, H-24); CIMS(NH_3) *m/z*: 648.

Treatment of **4** *with methyl boronic acid*. Treatment of **4** with excess $MeB(OH)_2$ in EtOAc followed a standard procedure [14]. EIMS (probe) 70 eV, *m/z* (rel. int.): 594 [M]$^+$ (0.2), 439 (12), 353 (29), 96 (100), 43 (67).

The thresholds of bitterness of **1** and **3** were determined by dissolving the compound (5 mg) in EtOH (0.5 ml) and diluting with mineral water to give solns containing 10, 5, 2, 1 and 0.5 ppm. Panellists tasted the solns, starting with mineral water, in order of increasing sample concentration, until bitterness was just perceived.

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