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POLYOXYGENATED *ENT*-KAURANES AND WATER-SOLUBLE CONJUGATES IN SEED OF *PHASEOLUS COCCINEUS*

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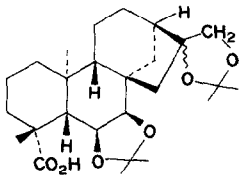
Key Word Index—*Phaseolus coccineus*; Leguminosae; *O*-isopropylidene derivatives: *ent*-6α,7α,16β,17-tetrahydroxykauranoic acid; water soluble conjugates; hydrolysis products; GC–MS identification.

Abstract—C-α and C-β, previously isolated from seed of *Phaseolus coccineus*, are shown respectively to be the *bis*-*O*-isopropylidene and the 16,17-mono-*O*-isopropylidene derivatives of *ent*-6α,7α,16β,17-tetrahydroxykauranoic acid. By GC–MS characterization of the products of acidic, basic and enzymatic hydrolysis, water soluble conjugates of the following compounds have been shown to occur in *P. coccineus* seed: GA_8, GA_{17}, GA_{20}, GA_{28}, *ent*-6α,7α,13-trihydroxykaurenoic acid, *ent*-6α,7α,17-trihydroxy-16βH-kauranoic acid, *ent*-6α,7α,16β,17-tetrahydroxykauranoic acid, 7β,13-dihydroxykaurenolide and abscisic acid.

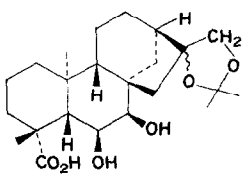
Two compounds, C-α and C-β, of undetermined structure were isolated from immature seed of *Phaseolus coccineus* by Durley *et al.* [1]. Evidence is now presented showing that C-α and C-β are respectively the 6,7,16,17-*bis*-*O*-isopropylidene and 16,17-mono-*O*-isopropylidene derivatives (**1**) and (**3**) of *ent*-6α,7α,16β,17-tetrahydroxykauranoic acid (**4**). Water soluble conjugates of the parent acid (**4**) and of other compounds have been detected in the BuOH–soluble extract from seed of *P. coccineus* by identification of their hydrolysis products.

The MS of the Me esters of C-α and C-β and of the MeTMS of C-β were published and discussed by Durley *et al.* [1]. From high resolution MS these authors concluded that the ion at *m/e* 447 was the M^+ ion, $C_{25}H_{37}NO_6$. However, the accurate mass measured for the *m/e* 447 ion was also consistent with the composition $C_{26}H_{39}O_6$ of an M^+-15 ion derived from an absent M^+, $C_{27}H_{42}O_6$. On this basis, the MS of MeC-α contains ions at M^+-58 (*m/e* 404), M^+-15-58 (*m/e* 389), *m/e* 58 and *m/e* 43 indicative [2] of an *O*-isopropylidene derivative. Furthermore the MS of MeC-α contained an ion at *m/e* 72, present [2] in the MS of the 1,2,4,5-*bis*-*O*-isopropylidene derivative of fructose and assigned [2] structure (**7**); this suggested that C-α contained a terminal *O*-isopropylidene function. This re-interpretation of the MS of MeC-α, together with the previously published [1] data indicated that C-α was the *bis*-isopropylidene derivative of a tetrahydroxykauranoic acid. This possibility was further strengthened by GC–MS comparison with the *bis*-isopropylidene derivative (**2**), prepared from *ent*-6α,7α,16α,17-tetrahydroxykauranoic acid (**5**) by Murofushi *et al.* [3] and provided by them. Although the two compounds had different R_Ts their MS were almost identical. C-α was therefore hydrolysed with acid in the presence of $(CH_2OH)_2$ and the resulting tetrahydroxy-acid was characterized by GC–MS as the MeTMS of *ent*-6α,7α,16β,17-tetrahydroxykauranoic acid (**4**) by direct comparison with an authentic [4] specimen. The MeTMS of the epimeric *ent*-6α,7α,16α,17-tetrahydroxykauranoic acid (**5**) isolated by Murofushi *et al.*[3] from seed of *Calonyction aculeatum*, had a similar MS but dif-

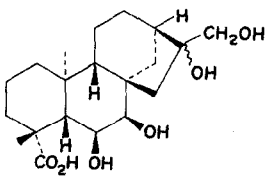
ferent GC retention time. C-α was regenerated from its hydrolysis product (**4**) on treatment with Me_2CO and TsOH.



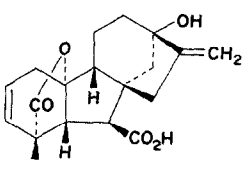
(1) 16α—O—
(2) 16β—O—



(3)



(4) 16 α — OH
(5) 16 β — OH



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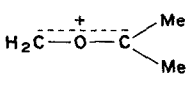

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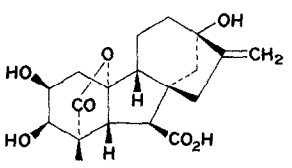
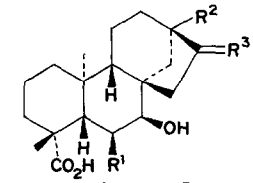


(8)

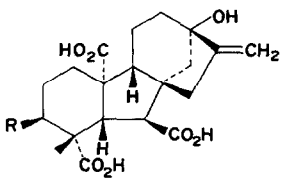
Compound C-β was characterized by Durley *et al.* [1] as a dihydroxy-monocarboxylic acid $C_{23}H_{36}O_6$. The MS of the MeTMS derivative contains fragment ions at M^+-15-58 (*m/e* 493), *m/e* 72, *m/e* 58 and *m/e* 43 characteristic of an *O*-isopropylidene derivative. The ion at *m/e* 147 indicated [5] the presence of a 1,2- or 1,3-diol in C-β and this was supported by the formation of an *n*-butyl boronate [6] from MeC-β which was characterized by GC–MS. Hydrolysis of C-β with acid in the presence of $(CH_2OH)_2$ gave *ent*-6α,7α,16β,17-tetrahydroxykauranoic acid (**4**), identified by GC–MS comparison of the MeTMS derivative with an authentic specimen [4]. From these facts it is concluded that C-β is the 16,17-mono-*O*-isopropylidene derivative (**3**) of *ent*-6α,7α,16β,17-tetrahydroxykauranoic acid (**4**). The alternative 6,7-mono-*O*-isopropylidene structure was excluded by the absence of strong ions at M^+-103 and *m/e* 103, characteristic [4] of tertiary $-CH_2OTMS$ groups, in the MS of the MeTMS derivative and by the presence of an intense ion at *m/e* 269, characteristic [7,8] of 6,7-*bis*-*O*-TMS-kauranoic acid Me esters.

The isopropylidene derivatives (**1**) and (**3**) are almost certainly formed during column chromatography of the EtOAc-soluble acids from *P. coccineus* seed from the free tetrahydroxy-acid (**4**) and the acetone used to adsorb the fraction onto the column and to elute it. Since the EtOAc-soluble acidic fraction, obtained by Durley *et al.* [1] was no longer available a search for the free tetrahydroxy-acid (**4**) was made in the extant BuOH-soluble fraction, isolated by the same workers [1]. GC–MS of the MeTMS-derivatized fraction showed the presence of only traces of the free acid (**4**) together with traces of GA_8. However, hydrolysis of the BuOH-soluble fraction with acid, base or a crude pectinase preparation gave many products. Those identified as the Me and/or MeTMS derivatives by GC–MS comparison with authentic samples are shown in Table 1. The acid hydrolysis products of the 13-hydroxy GAs and 13-hydroxykaurenes were identified as derivatives of the ring C/D rearrangement products (**15**) and abscisic acid (ABA) as the lactone (**16**). The compounds detected in the hydrolysates from the BuOH-soluble fraction are presumably present as water-soluble conjugates. Apart from GA_8, GA_{17} and GA_{20} which were identified as the free acids in the EtOAc fraction by Durley *et al.* [1] the compounds shown in Table 1 have not previously been detected in seed of *P. coccineus*. The tetrahydroxy-acid (**4**) and GA_8 (**8**) were the major products, present in much greater amounts after hydrolysis than before. Most of the conjugates are hydrolysed by base and may therefore be present as glycosidic esters [9]. GA_8 (**8**) is only released by enzymatic or acidic hydrolysis and is therefore probably present as the 2-*O*-β-glucoside previously detected in mature seed of *P. coccineus* by Schreiber *et al.* [10].

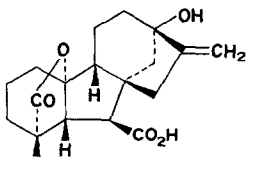
The enzymatic hydrolysis of the highly coloured, crude BuOH-soluble fraction gave a remarkably clean hydrolysate (see Fig. 1) and, in conjunction with base hydrolysis, provides a useful routine method of investigating water-soluble conjugates. The presence of ring C/D rearranged GA_8 and GA_{17} in the enzymatic hydrolysis is presumably due to their acid-catalysed formation during the isolation of the BuOH-soluble fraction.



(9) $R^1=R^2=OH$, $R^3=CH_2$
(10) $R^1=OH$, $R^2=H$, $R^3=\alpha H$, CH_2OH



(11) R=H
(12) R=OH



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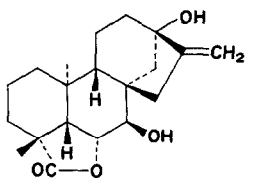

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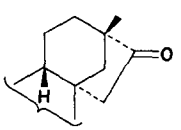

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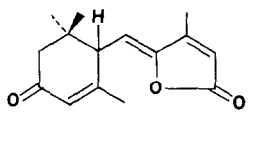

(16)

The presence of conjugates of *ent*-6α,7α,13-trihydroxykaurenoic acid (**9**) and 7β,13-dihydroxykaurenolide (**14**) shows that 13-hydroxylation of *ent*-kaurenes occurs in seed of *P. coccineus* and suggests that 13-hydroxylation may precede ring-contraction to GAs in this seed.

EXPERIMENTAL

For GC and GC–MS see Ref. 4. Me derivatives were prepared with CH_2N_2 and MeTMS derivatives were obtained by treating the Me derivatives with Sweeley's reagent [11] in a sealed tube with gentle warming when necessary.

Table 1. Hydrolysis products from water-soluble constituents of seed of *P. coccineus*

Product	Conditions of hydrolysis Acidic	Basic	Enzymatic
GA_{17} (**11**)	—	✓	✓
C/D rearranged GA_{17}	✓	—	✓
GA_{28} (**12**)	—	✓	—
C/D rearranged GA_{28}	✓	—	—
GA_8 (**8**)	—	—	✓
C/D rearranged GA_8	✓	—	✓
GA_{20} (**13**) C/D rearranged	✓	—	—
Rearranged ABA (**16**)	✓	—	—
(**9**)	—	✓	✓
C/D rearranged (**9**)	✓	—	✓
(**4**)	—	✓	✓
C/D rearranged (**14**)	✓	—	—
(**10**)	—	✓	✓

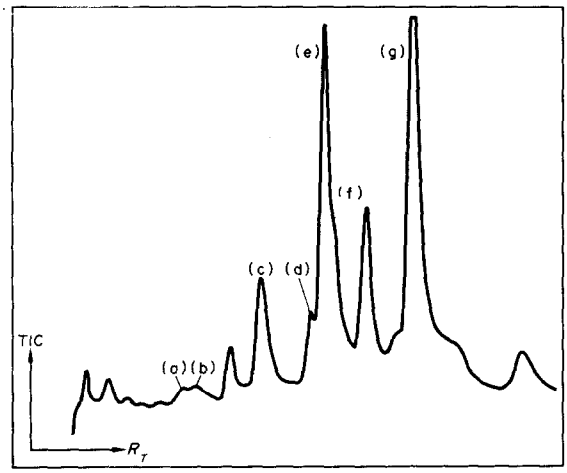

Fig. 1. GC–MS trace; MeTMS of enzyme hydrolysate of BuOH-soluble fraction; 2%-SE33, 205° programmed at 2°/min (a) GA_{17} (**11**) Me ring C/D rearrangement product; (b) GA_{17} (**11**) MeTMS; (c) MeTMS of compound (9); (d) GA_8 (**8**) MeTMS ring C/D rearrangement product; (e) GA_8 (**8**) MeTMS; (f) MeTMS of compound (**10**); and (g) MeTMS of tetraol (**4**).

Hydrolysis of C-α and C-β. MeC-α (*ca* 120 μg) or MeC-β (*ca* 120 μg) was treated with $(CH_2OH)_2$–H_2O (200 μl, 1:1) containing conc. H_2SO_4 (25 μl) for 24 hr at 18°. Dilution with H_2O (400 μl), extraction with EtOAc (3 × 1 ml) and evaporation of the dried extract gave a gum. In both cases this gummy product was derivatized and identified as the MeTMS derivative of *ent*-6α,7α,16β,17-tetrahydroxykauranoic acid (**4**) by GC–MS comparison with an authentic sample [4].

Hydrolysis of the BuOH-soluble fraction from seed of P. coccineus. (a) *Acidic.* A portion (50 mg) of the BuOH-soluble fraction, obtained by Durley *et al.* [1], was heated at 100° for 3 hr with N HCl (1 ml). Extraction with EtOAc and recovery from the extract gave a gum which was analysed by GC–MS of the Me and MeTMS derivatives. The ring C/D rearrangement products of the following compounds were identified by direct comparison of MS with published spectra [8]: GA_{17} (as Me ester), GA_{20} (as Me ester), *ent*-6α,7α,13-trihydroxykaurenoic acid (**9**) (as the MeTMS derivative) and 7β,13-dihydroxykaurenolide (**14**) (as the TMS derivative). Rearranged GA_8, GA_{28} and ABA were identified by GC–MS comparison of the following derivatives with authentic samples prepared by treatment of GA_8, GA_{28} or ABA with N HCl at 100° for 3 hr and derivatization of the product; rearranged GA_8 MeTMS, *m/e* 522 (M^+, 100%), 217 (45), 147 (40), 129 (25), 75 (23) and 73 (100): rearranged $MeGA_{28}$, *m/e* 436 (M^+, 21%), 404 (91), 376 (63), 358 (51), 348 (100), 344 (65), 326 (65), 316 (85), 199 (40), 194 (31) and 188 (51); and rearranged GA_{28} MeTMS, *m/e* 508 (M^+, 4%) 493 (9), 476 (4), 452 (30), 365 (32), 160 (25), 129 (100) and 73 (40); rearranged ABA lactone (16) *m/e* 246 (M^+, 1%), 204 (1), 190 (100), 162 (5), 147 (4), 134 (19), 119 (4), 106 (5), 96 (6), and 91 (9).

(b) *Alkaline.* The BuOH-soluble fraction (50 mg) was heated at 100° for 1 hr with 2N KOH (1 ml). The soln acidified to pH 2·5 with conc HCl was extracted with EtOAc (3 × 1 ml). The gum recovered from the EtOAc was GC–MS'd as the

MeTMS derivatives and identified by comparison with published MS. GA_{17} (**11**) [5], *ent*-6α,7α,13-trihydroxykaurenoic acid (**9**) [8], *ent*-6α,7α,16β,17-tetrahydroxykauranoic acid (**4**) [4] and *ent*-6α,7α,17-trihydroxy-16βH-kauranoic acid (**10**). GA_{28} (**12**) was identified as the MeTMS derivative by direct GC–MS comparison with an authentic sample; *m/e* 580 (M^+, 30%), 565 (14), 548 (13), 520 (12), 519 (12), 471 (15), 208 (95), 207 (100), 129 (75) and 73 (46).

(c) *Enzymatic.* The BuOH fraction (269 μg), "Boots Pectolytic Enzyme" (5·4 g) and KH_2PO_4 (0·68 g) in H_2O (100 ml) were incubated at 37° for 40 hr. The kieselguhr support was filtered off and the filtrate, after adjustment to pH 2 with conc HCl, was extracted with EtOAc (3 × 50 ml). The gum recovered from the EtOAc was derivatized and examined by GC–MS. The following compounds were identified from reference spectra: rearranged GA_{17}Me ester; GA_{17} MeTMS; Me *ent*-6α,7α,13-trihydroxykaurenoate TMS; rearranged Me *ent*-6α,7α,-13-trihydroxykaurenoate TMS; rearranged GA_8MeTMS; GA_8MeTMS; Me *ent*-6α,7α,17-trihydroxy-16βH-kauranoate TMS; and *ent*-6α,7α,16β,17-tetrahydroxykauranoate TMS.

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