Short Communication

Brassinolide-related New Bioactive Steroids from *Dolichos lablab* SeedJun BABA, Takao YOKOTA
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Since the discovery of brassinolide (1),¹⁾ new brassinolide analogs, dolicholide (2)²⁾ and castasterone (3)³⁾ have been isolated from immature seeds of *Dolichos lablab* and chestnut gall, respectively. The occurrence of 1 and 3 in chinese cabbage⁴⁾ and other plant sources⁵⁾ has also been demonstrated. Recently, two additional analogs were isolated from the *Dolichos* seeds, with which the present paper is concerned.

The crude extract of the immature seeds (34 kg) was first purified by silica gel chromatography. Elution with chloroform-methanol mixtures afforded two active fractions, namely the less polar one (eluted with a 95:5 mixture) and the polar one (eluted with a 90:10 mixture). The isolation of dolicholide from the latter fraction has already been reported.²⁾ The former fraction was successively purified by chromatographies on 60%-hydrated silica gel (benzene-ethyl acetate, 80:20), Sephadex LH-20 (70% aqueous ethanol) and high performance liquid chromatography on Partisil-5 (chloroform-isopropanol, gradient), and Develosil ODS-3 (acetonitrile-water, 45:55). The activity was monitored by a rice lamina inclination assay.⁶⁾ At the last step on the reversed phase HPLC, the activity was separated into two fractions and each of them was crystallized from aqueous acetonitrile to afford the new bioactive sterols 4, 50 μ g, mp 233~237°C and 5, 20 μ g (crude crystals), mp

204~208°C.*

4, named dolichosterone, gave rise to ions at m/z 463 ($M+1$) and 555 ($M+1+92$ (glycerin)) in the secondary ion MS. In the high resolution EIMS, where no trace of M^+ was observed, prominent ions at m/z 363.2584 ($C_{22}H_{35}O_4$) and 100.0892 ($C_6H_{12}O$, base peak) show that a C22~C23 bond fission occurred predominantly. The molecular formula $C_{28}H_{46}O_5$ is thus given to dolichosterone.

The 1H NMR spectrum is shown in Table I, compared with those of brassinolide (1), dolicholide (2) and castasterone (3).^{2,3)} Signals due to the side-chain protons are superimposable with those of dolicholide, indicating that 4 has a side-chain structure identical with that of dolicholide. This was confirmed by the double resonance experiment, in which a pair of methyl proton doublets at δ 1.08 and 1.11 (H_3 -26 and H_3 -27) collapsed into singlets by irradiating the 1H septet at δ 2.27 (H -25), while a 1H doublet at δ 3.63 (H -22) collapsed into a singlet by irradiating the 1H doublet at δ 4.03 (H -23). Signals due to the ring protons show a good correspondence with those of castasterone, indicating the presence of the same functionalities in the ring structure as castasterone. The absence of a lactone moiety

* The yields were roughly estimated by GC analysis of their bismethaneboronates.

TABLE I. ^1H NMR DATA (400 MHz, CDCl_3)

The chemical shifts are given in ppm downfield from TMS.

	1	2	3	4	5
Ring protons					
H ₃ -18	0.72 s	0.66 s	0.69 s	0.62 s	0.62 s
H ₃ -19	0.93 s	0.92 s	0.76 s	0.75 s	0.75 s
H-5	3.12 dd (5, 12 Hz)	3.11 dd (4, 12 Hz)	2.69 dd (4, 13 Hz)	2.68 dd (3, 13 Hz)	2.69 dd (4, 12 Hz)
H-2	3.72 br.	3.72 br.	3.77 br.	3.76 br.	*
H-3	4.03 br.s	4.02 br.s	4.06 br.s	4.05 br.s	4.05 br.s
H ₂ -7	4.09 m	4.09 m			
Side-chain protons					
Me (1)	0.85 d	0.96 d (H ₃ -21)	0.85 d	0.95 d (H ₃ -21)	0.93 d
Me (2)	0.91 d	1.08 d (H ₃ -26)	0.91 d	1.08 d (H ₃ -26)	1.06 d
Me (3)	0.95 d	1.11 d (H ₃ -27)	0.95 d	1.11 d (H ₃ -27)	1.14 d
Me (4)	0.97 d	—	0.97 d	—	1.75 d
H-25		2.26 septet		2.27 septet	2.78 septet
H-22	3.54 d (9 Hz)	3.62 d (8 Hz)	3.56 d (9 Hz)	3.63 d (8 Hz)	*
H-23	3.72 dd (2, 9 Hz)	4.03 d (8 Hz)	3.72 d (9 Hz)	4.03 d (8 Hz)	3.96 d (8 Hz)
H-28	—	5.04 s	—	5.03 s	5.52 q
H-28	—	5.07 s	—	5.06 s	—

* Not observed because of overlapping signals of contaminants.

in **4** was apparent because absorption due to oxymethylene protons (H₂-7), characteristic of lactone derivatives, was not observed and the H-5 signal shifted upfield compared with that of dolicholide.

Based upon the above evidence the structure **4**, (22*R*,23*R*)-2 α ,3 α ,22,23-tetrahydroxy-5 α -ergost-24(28)-en-6-one, is assigned to dolichosterone.

The ^1H NMR spectrum of **5** (named homodolichosterone) exhibited most of the absorptions observed in dolichosterone (**4**) (Table I). Instead of the exomethylene signals, however, signals ascribable to an ethylidene grouping were observed at δ 5.52 (1H, q, $J=7$ Hz) and δ 1.75 (3H, d, $J=7$ Hz). Since some of the important signals were not observed in the spectrum because of overlapping with the signals of contaminants, further purification was attempted. This, however, only resulted in a partial isomerization with respect to the double bond, which was evidenced by the ^1H NMR spectrum.

In the secondary ion MS of the re-purified

compound, ions were observed at m/z 477 ($M+1$) and 569 ($M+1+92$ (glycerin)). In the high resolution EIMS, ions due to a C22~C23 bond fission were observed at m/z 363.2518 ($\text{C}_{22}\text{H}_{35}\text{O}_4$) and 114.1061 ($\text{C}_7\text{H}_{14}\text{O}$). Thus the molecular formula $\text{C}_{29}\text{H}_{48}\text{O}_5$ was allocated to homodolichosterone. Unusually abundant ions due to a C20~C22 bond fission were observed at m/z 334.2523 ($\text{C}_{21}\text{H}_{34}\text{O}_3$) and 125.0977 ($\text{C}_8\text{H}_{13}\text{O}$, presumably a side-chain fragment with loss of H_2O), which might be indicative of a double bond migration.

Thus the structure **5**, (22*R*,23*R*)-2 α ,3 α ,22,23-tetrahydroxy-5 α -stigmast-24(28)-en-6-one, is tentatively assigned to homodolichosterone. The orientation of the methyl group in the ethylidene moiety, however, remains to be determined.

Recently a brassinolide analog carrying an ethyl group at C24 and those without a substituent at C24 have been shown to be present in higher plants.⁵⁾ Together with these findings, our paper demonstrates the occurrence in higher plants of a series of biogenetically-

related brassinolide analogs, especially with respect to alkyl substituents at C24.

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