Tetrahedron Letters,Vol.23,No.47,pp 4965-4966,1982 0040-4039/82/474965-02$03.00/0
Printed in Great Britain

A NEW STEROIDAL LACTONE WITH PLANT GROWTH-REGULATORY ACTIVITY FROM DOLICHOS LABLAB SEED

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Abstract A new plant growth-promoting sterol was isolated from immature seeds of Dolichos lablab The structure was elucidated as (22R, 23R)-2α, 3α, 22, 23-tetrahydroxy-B-homo-7-oxa-5α-ergost-24(28)-en-6-one (1)

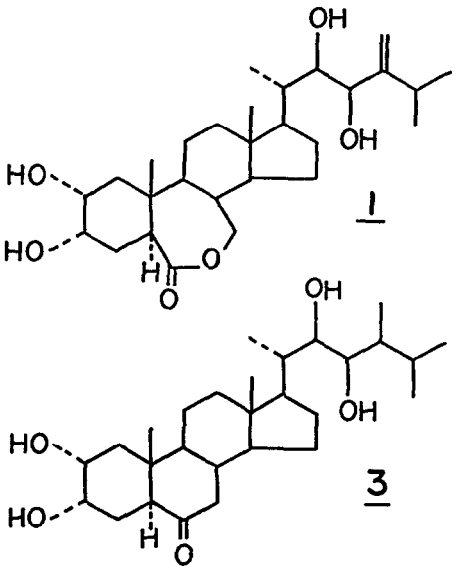
Plant growth-promoting phytosterols, namely brassinolide (2)[1] and castasterone (3)[2] have recently been isolated from rape pollen and chestnut insect gall, respectively We report herein the isolation and structure elucidation of a new steroidal plant growth-promotor contained in seeds of Dolichos lablab (Leguminosae)

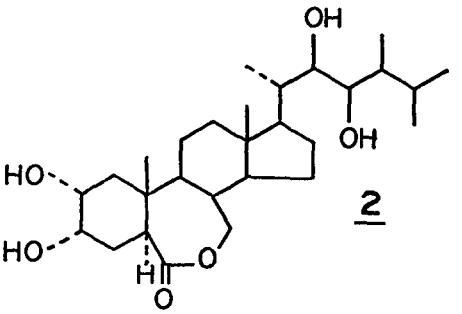
The isolation procedure was guided by the rice lamina inclination assay[3] which is very sensitive for brassinolide and related analogs. The aqueous residue obtained after concentrating the methanol extract of the immature seeds was subjected to solvent partitionings The benzene- and chloroform-extractable fractions were combined and again partitioned between hexane and 80% aqueous methanol. The latter was successively purified by chromatographies on silica gel (chloroform-methanol, 90 10), 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 active fraction was crystallized from aqueous acetonitrile to afford a new biologically-active sterol (1), mp. 234-238°C, which was named dolicholide. The yield was 160 μg from 34 kg of the seeds

In the secondary ion MS, 1 gave rise to prominent ion peaks at m/z 479 (M + 1) and m/z 571 (M + 1 + 92 (glycerin)) In the high resolution EIMS, 1 produced strong ions at m/z 379 2431 (rel int. 47%, $C_{22}H_{35}O_5$) and 100 0878 (base peak, $C_6H_{12}O_1$), which are suggested to be derived through a C_{22}-C_{23} bond fission characteristic of brassinolide and castasterone Thus the molecular formula $C_{28}H_{46}O_6$ is given to dolicholide.

In the 400 MHz NMR in $CDCl_3$, resonances due to the protons in the A and B rings were observed at δ 0 92(3H, s, H_3-19), 3 11 (1H, dd, J=4, 12 Hz, H-5), 3.72 (1H, br , $W_{1/2}$=22 Hz, axial H-2), 4 02 (1H, overlapped br.s, equatorial H-3) and 4.09 (2H, m, H_2-7). These signals are superimposable with those reported for brassinolide (2)[2], indicating dolicholide has the same structure and stereochemistry in the A and B rings as brassinolide This was supported by extensive decoupling experiments which revealed the following coupling sequence equatorial H-1 (δ 1.86) — H-2 — H-3 — H_2-4 (δ 1 96, 2 15) — H-5.

Signals due to the side-chain protons were assigned as follows δ0.96 (3H, d, J=7 Hz, H_3-21), 1 08 (3H, d, J=8 Hz, H_3-26 or -27), 1 11 (3H, d, J=8 Hz, H_3-27 or -26), 1 47 (1H, overlapped m, H-20), 2 26 (1H, septet, H-25), 3 62 (1H, d, J=8 Hz, H-22), 4 03 (1H, d, J=8 Hz, H-23), 5 04





(1H, s; H-28), 5.07 (1H, s; H-28). These assignments were based on the following double-resonance experiments Irradiation of H-25 sharpened one (δ 5.04) of olefinic methylene protons and also caused collapse of a pair of the methyl-proton signals (δ 1 08, 1.11) into singlets. When H-23 was irradiated a signal due to one of olefinic protons (δ 5.07) was sharpened and a signal due to H-22 collapsed into a singlet The stereochemistry of three chiral centers (C20, C22 and C23) should be considered identical with brassinolide because the coupling constants between H-22 and H-23 (J=8 Hz) and between H-22 and H-20 (J≃0 Hz) were nearly the same as those of brassinolide.

Based on the above evidences, as well as biogenetic considerations, the structure of 1 is deduced for dolicholide. The fact that dolicholide gave rise to an ion of m/z 100 as the base peak in the EIMS can be easily explained from the allyl alcohol moiety in the structure 1.

The biosynthetic relation between dolicholide, brassinolide and castasterone will be a subject of interest, although the latter two are nor yet found in the Dolichos seed. The biological activity of dolicholide in the rice-lamina inclination assay was about one-tenth of that of brassinolide.

Acknowledgements The authors wish to thank Mr. K. Furihata and Dr. H. Seto, Institute of Applied Microbiology, for the measurement of ^{1}H NMR

References and Notes

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(Received in Japan 12 July 1982)