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CASTASTERONE, A NEW PHYTOSTEROL WITH PLANT-HORMONE POTENCY, FROM CHESTNUT INSECT GALL

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Abstract: A sterol which has strong potency to inclinate rice lamina was isolated from the insect gall of the chestnut tree. The structure was determined to be (22R, 23R, 24S)-2α, 3α, 22, 23-tetrahydroxy-24-methyl-5α-cholestan-6-one (1).

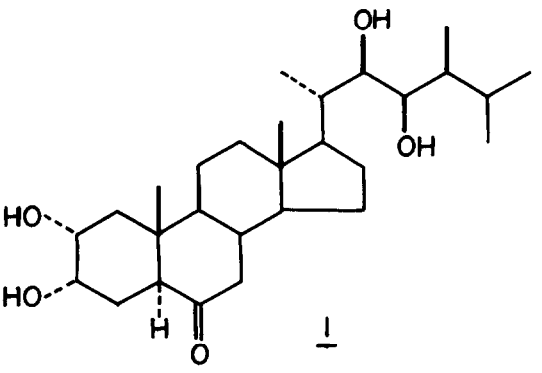
Brassinolide (2) isolated from rape pollen is a naturally-occurring steroidal plant-growth promotor. Recently brassinolide and its synthetic analog (homobrassinolide) were found to possess strong activity[2] in the rice-lamina inclination assay[3]. Plant extracts from a variety of plants have been demonstrated by Japanese workers[4] to exhibit strong activity in this sensitive bioassay, suggesting that brassinolide or related compounds might be widely distributed in the plant kingdom.

We have isolated a new sterol named castasterone, highly active in the rice-lamina inclination test, from the insect galls of the chestnut tree (*Castanea* spp). This paper describes the structure of this sterol which is considered closely related to brassinolide.

The methanol extract of the gall (40 kg) was partitioned between benzene and water. The benzene fraction was again partitioned between hexane and 90% aqueous methanol. The methanol fraction was successively purified by chromatographies on silica gel (chloroform-methanol 95:5; ethyl acetate), on Sephadex LH-20 (70% aqueous ethanol) and by high performance liquid chromatography on Partisil 5 (chloroform-isopropanol, gradient) and Develosil ODS-3 (acetonitrile-water 1:1), affording 95 μg of castasterone (1), which was crystallized from aqueous acetonitrile, mp. 259-261°C.

In the fast atom bombardment MS, an ion at m/z 465 (M + 1, base peak) was observed along with ions at m/z 447 (M + 1 - 18) and 429 (M + 1 - 36)[5]. The molecular formula $C_{28}H_{48}O_5$ was deduced from the compositions of the fragment ions (e.g. m/z 393 ($C_{23}H_{37}O_5$) and 364 ($C_{22}H_{36}O_4$)) determined in the high-resolution, electron-impact MS (Table 1) where only a trace of M^+ was observed. As shown in Table 1, fragment ions formed through breakdown of the side-chain and D ring (a ∿ f) indicate the presence of 22-hydroxyl, 23-hydroxyl and 24-methyl groups in the side chain, and no functionality in the D ring. A fragment ion characteristic of 6-keto steroid was observed at m/z 155 ($C_8H_{11}O_3$)[6]. This ion indicates the presence of two hydroxyl groups in the A ring.

The CD curve (methanol) showed a negative maximum (θ = -5475) at 292 nm, consistent with the data of 5α-cholestan-6-one but not with that of its 5β-epimer[7], thus establishing the



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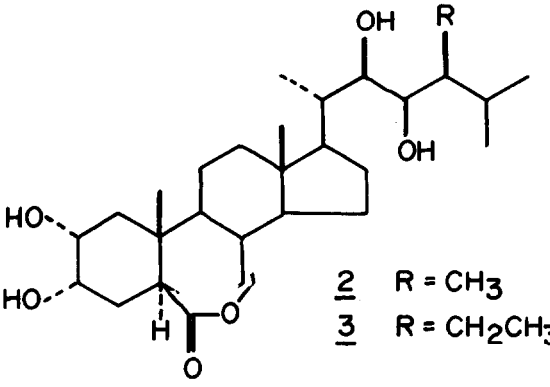
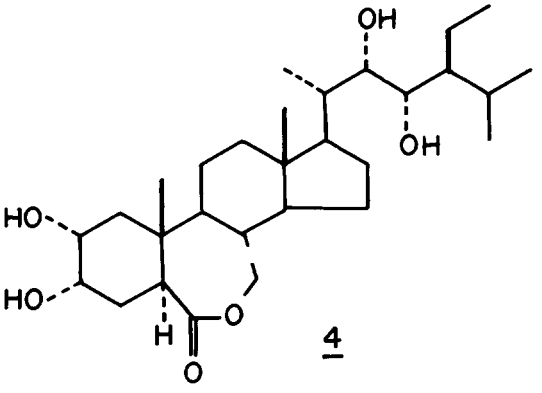


2 R = CH_3

3 R = CH_2CH_3



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presence of trans-fused A/B ring as well as of the 6-keto group. The absence of a lactone linkage characteristic of brassinolide was established by the ^{1}H NMR which lacks an absorption due to oxidized-C7 methylene protons (Table 2).

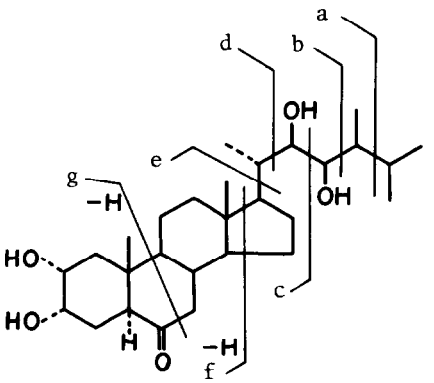
The presence of both axial and equatorial hydroxyl groups was shown by the ^{1}H NMR absorptions at δ 3.77 ($W_{1/2}$ = 23 Hz) and 4.06 ($W_{1/2}$ = 8 Hz). These signals were also encountered in the synthetic brassinolide (2)[8, 9] with nearly identical chemical shifts and shapes, strongly suggesting the presence of C2α and C3α hydroxyl groups (Table 2). The low-field chemical shift of the C5 H (δ 2.69) is explainable by a 1,3-diaxial relationship between the C5 H and C3α hydroxyl group.

A pair of doublets at δ 3.56 (J = 9 Hz) and 3.72 (J = 9 Hz) are suggestive of the presence of 22R, 23R-dihydroxyl groups, since these signals are observed in brassinolide (2) and (22R, 23R)-homobrassinolide (3)[10] with similar chemical shifts and coupling constants, but they are found as a 2H multiplet centered at δ 3.60 in (22S, 23S)-homobrassinolide (4)[9, 11]. The methyl groups in the side chain appeared at δ 0.85, 0.91, 0.95 and 0.97, whose chemical shifts were exactly identical with those of brassinolide (2). Thus the side chain structure in castasterone is the same as that of brassinolide.

Based on the above evidences the structure (1) is assigned to castasterone. Final confirmation for this structure was obtained by the fact that castasterone showed identical

Table 1. Fragment ions of castasterone in the high-resolution, electron-impact MS (70 eV)

Cleavage site	Ions
a	403 (0.4)*, 385 (0.3)*
b	393 (3), 375 (2)*, 357 (2)*
c	364 (100), 363 (35), 362 (36)
	345 (59)*, 346 (42)*, 327 (36)*
d	315 (7)*
e	305 (5), 287 (17)*, 269 (10)*
f	263 (11), 245 (13)*, 227 (5)*
g	155 (5)



Figures in the brackets indicate relative intensities (base peak = 100).
The compositions of the ions were established within 7 millimass errors.
The compositions of the ions at m/z 403, 385 and 357 were not determined.
*Ions formed by additional loss of H_2O or 2 × H_2O.

Table 2. ^1H NMR data (400 MHz, $CDCl_3$)

	1	2	3	4
C18 CH_3	0.69 s	0.72 s	0.71 s	0.74 s
C19 CH_3	0.76 s	0.93 s	0.93 s	0.93 s
CH_3	0.85 d	0.85 d	0.91 d	0.88 d
CH_3	0.91 d	0.91 d	0.96 t	0.95 d
CH_3	0.95 d	0.95 d	0.96 d	0.96 t
CH_3	0.97 d	0.97 d	0.98 d	1.03 d
C5 H	2.69 dd (4, 13 Hz)	3.12 dd (5, 12 Hz)	3.12 dd (4, 12 Hz)	3.11 dd (4, 12 Hz)
C22(23) H	3.56 d (9 Hz)	3.54 d (9 Hz)	3.58 d (9 Hz)	3.60 m
C23(22) H	3.72 d (9 Hz)	3.72 dd (2, 9 Hz)	3.72 d (9 Hz)	
C2 H	3.77 br. ($W_{1/2}$= 23 Hz)	3.72 br. (overlapped)	3.72 br. (overlapped)	3.73 br. ($W_{1/2}$= 23 Hz)
C3 H	4.06 br.s ($W_{1/2}$= 8 Hz)	4.03 br.s ($W_{1/2}$= 10 Hz)	4.03 br.s ($W_{1/2}$= 10 Hz)	4.03 br.s ($W_{1/2}$= 8 Hz)
C7 H_2		4.09 m	4.10 m	4.09 m

The chemical shifts are given in ppm downfield from TMS.

properties (^{1}H NMR, MS, CD, mp., TLC[12]) with those of an authentic compound prepared by alkaline hydrolysis of the corresponding synthetic tetraacetate[9] supplied by Professor K. Mori.

From the structural outlook, castasterone can be regarded as a biosynthetic precursor for brassinolide. Even a trace of brassinolide, however, was not detectable in the extract of the chestnut gall, indicating that castasterone itself also might bear a physiological function in this plant tissue. The activity of castasterone in the rice (cv. Koshihikari)-lamina inclination test was observed at the concentration as low as 0.0001 ppm and was estimated to be half to quarter of that of synthetic brassinolide over the concentration range of between 0.0001 ppm to 0.01 ppm.

Castasterone must be of plant origin, since the extract of the larvae collected from the gall tissus has been reported to fail to show activity in the rice-lamina inclination test[13].

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