Synthesis of Castasterone and its 22*S*,23*S*-Isomer: Two Plant Growth Promoting Ketones

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Castasterone (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (1) and its (22*S*,23*S*)-isomer have been synthesized from (20*S*)-6β-acetoxy-3α,5-cyclo-5α-pregnane-20-carbaldehyde. The side chain with the correct stereochemistry was constructed *via* a Claisen rearrangement and the nuclear functionalities were introduced by osmilation of the intermediate obtained by opening of the cyclopropane ring with hydrochloric acid and dehydrohalogenation.

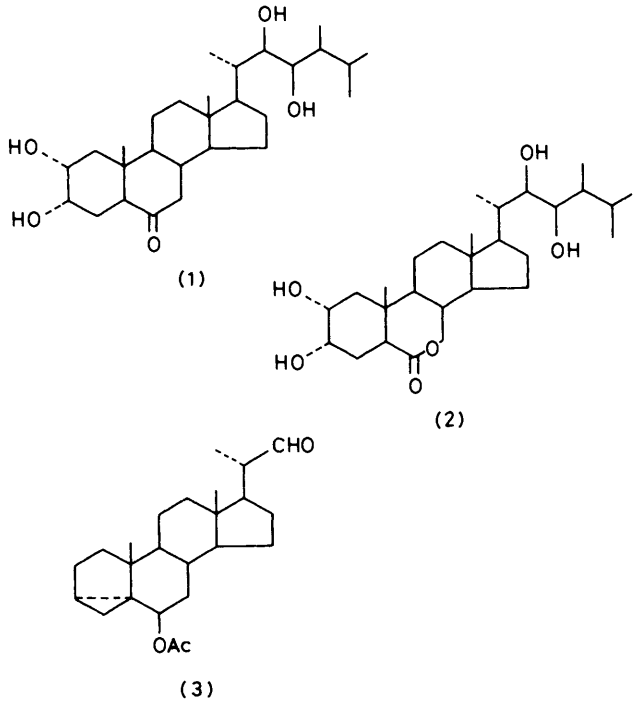
Castasterone (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one) (1) is a sterol highly active in the rice-lamina inclination test recently isolated [1] from the insect galls of the chestnut tree (*Castanea* species) (90 μg from 40 kg of the galls). However (1) appears to be of plant origin since the extract of the larvae collected from the gall tissues has been reported to fail to show activity in the rice-lamina inclination test.[1] This sterol is considered as a biosynthetic precursor of brassinolide (2), a new plant growth-promoting steroidal lactone isolated from rape pollen [2] (40 mg from 40 kg) in 1979. However castasterone itself might bear a physiological function in plant tissues.

The biological activities and scarcity of (1) and (2) have stimulated research on the synthesis of brassinolide [3a,d] and its analogues [4] many of which show extremely strong activity in stimulating the lamina inclination of rice plants. However until now only a synthesis of castasterone has been reported.† As part of an investigation on phytosteroids,[5] we have devised a synthetic approach to (1) and its isomer (13).

The dihydroxy side chain of (1) was constructed *via* the method developed by Sucrow and his co-workers [6] and adapted by us for the synthesis of oogonilo [6a] and stellasterol [6b] side chains. The method allows the construction of the chiral centre at the C-24 in a predictable way from a *Z* allylic C-22 alcohol *via* a Claisen rearrangement.[7] (20*S*)-6β-Acetoxy-3α,5-cyclo-5α-pregnane-20-carbaldehyde (3) (prepared by ozonolysis of cyclostigmasterol acetate [8]) was chosen as starting material for the elaboration of the side chain, since the skeletal functions can be introduced *via* the elaboration of the cyclopropane ring.

The aldehyde (3) reacted with 3-methylbutynylmagnesium bromide to give a 3 : 2 mixture (65% yield) of two acetylenic alcohols (4a) and (4b) whose C-24 stereochemistry was established by hydrogenation and removal of the acetoxy-protecting group to afford the known (5a) and (5b) which are readily differentiated by their melting points.[9]

Having established its configuration, we next partially hydrogenated the 22*S*-alcohol (4b) over Lindlar catalyst to give the *Z* allylic alcohol (6) which was subjected to Claisen rearrangement with triethyl orthoacetate in refluxing xylene to provide in good yield the Δ^{22}-unsaturated ester (7) the C-24 stereochemistry of which follows from the previously established one of its 22-hydroxy-precursor (6). Di-isobutylaluminium hydride reduction [10] of (7) at −78 °C in toluene affords in good yield the 29-aldehyde (8). This compound was quantitatively decarbonylated by tris(triphenylphosphine)-chlororhodium treatment [11] to (22*E*,24*S*)-3α,5-cyclo-5α-ergost-22-en-6β-ol (9). Compound (9) possesses the C-24 methyl group with the correct stereochemistry of castasterone and a Δ^{22}-double bond useful for the introduction of C-22 and C-23 hydroxy-groups.

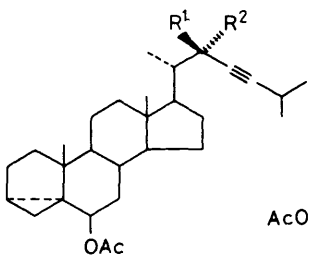


In order to introduce the nuclear functionalities compound (9) was oxidized with Jones reagent to the ketone (10) which was quantitatively transformed into (22*E*,24*S*)-3β-chloro-5α-ergostan-6-one (11) by treatment at room temperature with hydrochloric acid in acetic acid.[12] Dehydrohalogenation of (11) in dimethylformamide which contained lithium bromide at reflux for 30 min, gave (22*E*,24*S*)-ergosta-2,22-dien-6-one (12).‡ The ^{1}H n.m.r. spectrum of (12) supports the assigned structure and the C-24*S*-configuration. In fact the chemical shift of the 21-CH_3 signal which for the 24*R*-isomer resonates at δ 1.006, is shielded to 0.997 p.p.m., as expected.[13]

Treatment of (12) with 2 molar equivalents of osmium tetraoxide in dry diethyl ether and a trace of pyridine afforded a mixture of two tetrahydroxylated compounds which were

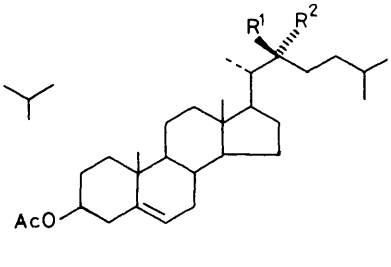
† During the preparation of this manuscript a synthesis of castasterone appeared (see ref. 3*d*) in which it was obtained mixed with three isomers.

‡ This route to introduce the Δ^2 double bond, in our hands, proved more suitable than that involving the regeneration of a 3β-hydroxy-group, tosylation, and dehydrotosylation.[3a-d]



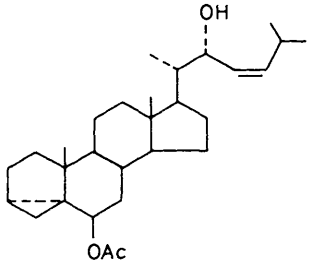
(4)

	R^1	R^2
a;	OH	H
b;	H	OH

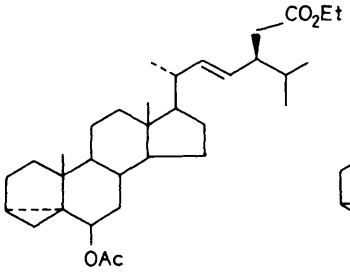


(5)

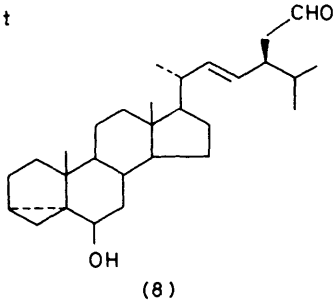
	R^1	R^2
a;	OAc	H
b;	H	OAc



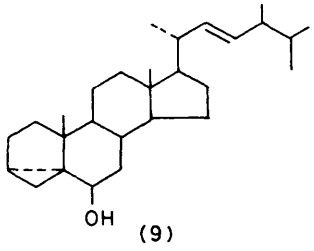
(6)



(7)



(8)



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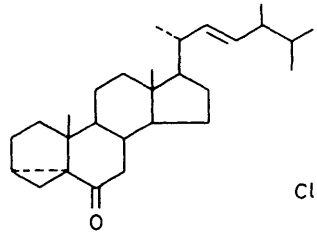

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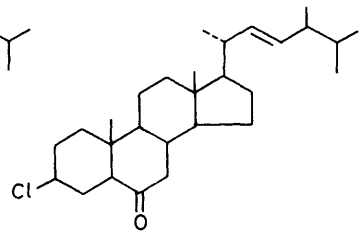
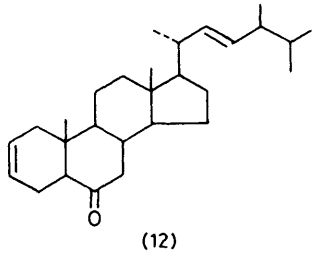
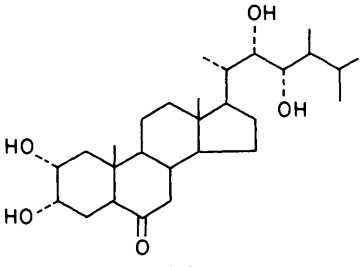


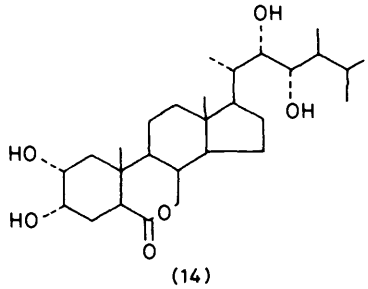
(11)



(12)



(13)



(14)

separated by chromatography. The less polar one (the minor component of the mixture, 15—20%) showed analytical figures corresponding to casterone (1).[1] To the more abundant, more polar component of the mixture was then attributed the structure of (22*S*,23*S*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (13). Its physicochemical properties compare well with those reported.[3d]

The ratio of the two isomers (1) and (13) obtained in our reaction is unfavourable for obtaining casterone as observed by Thompson *et al.* in the osmilation of cyclo-ketones derived from 22-dehydrocampesterol in mixture with the isomeric one derived from brassicasterol.[3d]

Because Thompson *et al.*[3d] have been able to transform (1) and (13) into brassinolide (2) and its 22*S*,23*S*-isomer (14), the present synthesis of (1) and (13) represents an alternative route to these steroidal lactones.

Experimental

All m.p.s are uncorrected. I.r. spectra were recorded for solution in chloroform or for Nujol mulls.

^{1}H N.m.r. spectra were recorded on a Varian XL-100 spectrometer in [^{2}H]chloroform solutions with $SiMe_4$ as internal standard. Mass spectra were recorded on a Varian 112 S mass spectrometer (direct inlet). The progress of all reactions and column chromatography (silica 230—400 mesh) was monitored by t.l.c. on E. Merck silica gel HF_{254} plates visualized by spraying with 70% sulphuric acid followed by heating.

(20S)-6β-*Acetoxy*-3α,5-*cyclo*-5α-*pregnane*-20-*carbaldehyde* (3).—Compound (3) was obtained by acetylation at room temperature with acetic anhydride and pyridine of the cyclostigmasterol and successive ozonolysis.[8]

(22R)- and (22S)-6β-*Acetoxy*-3α,5-*cyclo*-5α-*cholest*-23-*yn*-22-*ol* (4a) *and* (4b).—A solution of ethylmagnesium bromide was prepared from ethyl bromide (3.9 ml) and magnesium (1.2 g) in diethyl ether (10 ml). The solution was added to 3-methylbut-1-yne (6.5 ml) in tetrahydrofuran (15 ml) at −15 °C (argon atmosphere). The resulting mixture was stirred at −15 °C for 30 min and then allowed to warm to room temperature. After 45 min the solution was added dropwise to a stirred solution of the aldehyde (3) (2 g) in freshly dried tetrahydrofuran (50 ml) at 0 °C during 5 min. The mixture was stirred for 45 min and then it was treated with aqueous ammonium chloride (excess) before extraction with diethyl ether. The

dried organic layer was evaporated under reduced pressure to give the crude adduct which was chromatographed to give (22R)-6β-*acetoxy*-3α,5-*cyclo*-5α-*cholest*-23-*yn*-22-*ol* (4a) (780 mg) as an oil; δ 0.30—0.60 (3 H, m), 0.72 (3 H, s), 1.00 (3 H, s), 2.01 (3 H, s, OAc), 4.40 (1 H, m, 22-H), and 4.5 (1 H, m, 6-H); *m/z* 440 (Found: C, 79.1; H, 10.05. $C_{29}H_{44}O_3$ requires C, 79.04; H, 10.06%).

The more polar epimer (22S)-6β-*acetoxy*-3α,5-*cyclo*-5α-*cholest*-23-*yn*-22-*ol* (4b) (505 mg) was an oil, δ 0.3—0.6 (3 H, m), 0.72 (3 H, s), 1.00 (3 H, s), 2.00 (3 H, s, OAc), 4.40 (1 H, m, 22-H), and 4.5 (1 H, m, 6-H); *m/z* 440 (Found: C, 79.15; H, 10.1. $C_{29}H_{44}O_3$ requires C, 79.04; H, 10.06%).

(22S-)- *and* (22R)-22-*Hydroxycholesterol Diacetate* (5a) *and* (5b).— The acetylene (4a) (80 mg) dissolved in methanol (7 ml) was stirred with 60 mg of PtO_2 in a hydrogen atmosphere at room temperature and pressure for 2 h. The catalyst was filtered off and the solvent eliminated. The residue was dissolved in diethyl ether (1 ml) and acetic anhydride (1 ml) and freshly distilled boron trifluoride–diethyl ether (0.2 ml) was added at 0 °C. The mixture was stirred for 30 min, and then worked up to afford (22*S*)-22-hydroxycholesterol diacetate (5a) which was crystallized from methanol; it had m.p. 142—144 °C; δ 0.69 (3 H, s, 18-H), 1.01 (3 H, s, 19-H), 2.02 (6 H, s, 2 × OAc), 4.59 (1 H, m, 3-H), 4.94 (1 H, m, 22-H), and 5.38 (1 H, m, 6-H) (lit.,[16] 142—144 °C; and similar ^{1}H n.m.r. spectrum) (Found: C, 76.8; H, 10.8. Calc. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.37%).

The acetylene (4b) (100 mg) was converted into the corresponding (22*R*)-22-hydroxycholesterol diacetate (5b) in the same way as described above to yield a product (70 mg) which was crystallized from methanol; it had m.p. 98—99 °C; δ 0.68 (3 H, s, 18-H), 1.01 (3 H, s, 19-H), 2.02 (6 H, s, 2 × OAc), 4.57 (1 H, m, 3-H), 4.85 (1 H, m, 22-H), and 5.33 (1 H, m, 6-H) (lit.,[14] m.p. 96—98 °C, and similar ^{1}H n.m.r.) (Found: C, 76.8; H, 10.8. $C_{31}H_{50}O_4$ requires C, 76.50; H, 10.37%).

(23Z,22R)-6β-*Acetoxy*-3α,5-*cyclo*-5α-*cholest*-23-*en*-22-*ol* (6).—Hydrogenation of the alcohol (4b) (500 mg) over Lindlar catalyst (160 mg) in ethyl acetate (15 ml) for 2 h followed by crystallization from methanol gave (23Z,22R)-6β-*acetoxy*-3α,5-*cyclo*-5α-*cholest*-23-*en*-22-*ol* (6), m.p. 98—103 °C (from hexane); δ 0.30—0.60 (3 H, m), 0.70 (3 H, s), 1.01 (3 H, s), 2.00 (3 H, s, OAc), 4.40 (2 H, overlapping 22-H and 6-H), 5.30 (1 H, dd, *J* 8 and 11 Hz, 24-H), and 5.40 (1 H, dd, *J* 7 and 11 Hz, 23-H); *m/z* 442 (Found: C, 78.6; H, 10.5. $C_{29}H_{46}O_3$ requires C, 78.73; H, 10.41%).

Ethyl (22E,24S)-6β-*Acetoxy*-3α,5-*cyclo*-5α-*stigmast*-22-*en*-29-*oate* (7).—The *Z* allylic alcohol (6) (410 mg) was heated under reflux in xylene (15 ml) with ethyl orthoacetate (4 ml) and propionic acid (100 ml) with continuous removal of ethanol. After 3 h the solution was washed with saturated aqueous sodium hydrogencarbonate and once with water and then dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was crystallized from methanol to give *ethyl* (22E,24S)-6β-*acetoxy*-3α,5-*cyclo*-5α-*stigmast*-22-*en*-29-*oate* (7) (440 mg) as an oil; δ 0.30—0.60 (3 H, m), 0.72 (3 H, s,) 1.01 (3 H, s), 2.00 (3 H, s, OAc), 2.28 (2 H, m, 28-CH_2), 4.08 (2 H, q, OCH_2, *J* 7 Hz), 4.50 (1 H, m, 6-H), and 5.25 (2 H, m, 22-H and 23-H); *m/z* 512 (Found: C, 77.4; H, 10.25. $C_{33}H_{52}O_4$ requires C, 77.34; H, 10.16%).

22E,24S)-6β-*Hydroxy*-3α,5-*cyclo*-5α-*stigmast*-22-*en*-29-*al* (8).—The ester (7) (420 mg) in absolute toluene (15 ml) was cooled to −70 °C and di-isobutylaluminium hydride (235 mg in 1 ml of toluene) was added under nitrogen. The solution was kept at −70 °C for 2 h before ethyl acetate (0.5 ml) was added. The solution was allowed to warm to room temperature and then poured into saturated aqueous ammonium chloride. Work-up followed by chromatography afforded (22E,24S)-6β-*hydroxy*-3α,5-*cyclo*-5α-*stigmast*-22-*en*-29-*al* (8) (360 mg) as an oil; $\nu_{max.}$ 3 335, 2 720, and 1 720 cm^{-1}; δ 0.30—0.60 (3 H, m), 0.70 (3 H, s), 1.00 (3 H, s), 2.32 (3 H, m, 24- and 28-H), 3.20 (1 H, m, 6-H), 5.10—5.30 (2 H, m, 22- and 23-H), and 9.70 (1 H, m, CHO); *m/z* 426 (Found: C, 81.6; H, 10.65. $C_{29}H_{46}O_2$ requires C, 81.73; H, 10.82%).

(22E,24S)-3α,5-*Cyclo*-5α-*ergost*-22-*en*-6β-*ol* (9).—The aldehyde (8) (500 mg) was dissolved in degassed toluene (10 ml) and was refluxed under nitrogen in the presence of tris(triphenylphosphine)chlororhodium (500 mg) for 3 h. The mixture was filtered through a pad of silica gel G-Celite and the solvent was removed under reduced pressure. The residue was crystallized from methanol to yield (22E,24S)-3α,5-*cyclo*-5α-*ergost*-22-*en*-6β-*ol* (9) an oil, $\nu_{max.}$ 3 015 and 3 060 cm^{-1}; 0.30—0.60 (3 H, m), 0.72 (3 H, s), 1.02 (3 H, s), 3.24 (1 H, m, 6-H), and 5.10—5.30 (2 H, m, 22- and 23-H); *m/z* 398 (Found: C, 84.3; H, 11.5. $C_{28}H_{46}O$ requires C, 84.35: H; 11.63%).

(22E,24S)-3α,5-*Cyclo*-5α-*ergost*-22-*en*-6-*one* (10).—Jones reagent [14] was added at −15 °C to a solution of (9) (500 mg) in acetone (25 ml) until present in an excess; the excess was then discharged with methanol (1 ml). After work-up the crude product was crystallized from methanol to yield (22E,24S)-3α,-5-*cyclo*-5α-*ergost*-22-*en*-6-*one* (10) (460 mg), m.p. 105—108 °C (from methanol); $\nu_{max.}$ 1 695 cm^{-1}; δ 0.30—0.60 (3 H, m), 0.72 (3 H, s), 1.00 (3 H, s), and 5.10—5.30 (2 H, m, 22- and 23-H); *m/z* 396 (Found: C, 84.5; H, 11.3. $C_{28}H_{44}O$ requires C, 84.78; H, 11.18%).

(22E,24S)-3β-*Chloro*-5α-*ergost*-22-*en*-6-*one* (11).—To the ketone (10) (600 mg) dissolved in acetic acid (12 ml) was added hydrochloric acid (0.8 ml of a 37% solution) at 25 °C. After 10 min a crystalline product formed which was filtered off and washed with water to yield (22E,24S)-3α-*chloro*-5α-*ergost*-22-*en*-6-*one* (11) (500 mg), m.p. 150—151 °C (from methanol); δ 0.70 (3 H, s), 0.98 (3 H, s), and 5.15—5.30 (2 H, m, 22- and 23-H); *m/z* 396 (M^+ − HCl) (Found: C, 77.2; H, 10.5; Cl, 8.2. $C_{28}H_{45}ClO$ requires C, 77.17; H, 10.44; Cl, 8.16%).

(22E,24S)-5α-*Ergosta*-2,22-*dien*-6-*one* (12).—The chloroketone (11) (350 mg) was dissolved in dimethylformamide (15 ml) and lithium bromide (450 mg) was added. The mixture was refluxed for 1 h, then poured into water and extracted with diethyl ether. Work-up followed by chromatography afforded (22E,24S)-5α-*ergosta*-2,22-*dien*-6-*one* (12) (230 mg), m.p. 111—112 °C (from methanol); δ (200 MHz) 0.677 (3 H, s), 0.702 (3 H, s), and 0.997 (3 H, d, *J* 6.5, 21-H_3); *m/z* 396 (Found: C, 84.6; H, 11.6. $C_{28}H_{44}O$ requires C, 84.78; H, 11.18).

(22R,23R,24S)-2α,3α,22,23-*Tetrahydroxy*-5α-*ergostan*-6-*one* (1).—Osmium tetraoxide (360 mg) was added to a solution of the ketone (12) (250 mg) in diethyl ether (7 ml) containing pyridine (0.5 ml); the mixture was then allowed to stand at room temperature in the dark for 12 h. Methylene chloride was added and pyridine was eliminated by washing with dilute hydrochloric acid. The organic solution was then shaken with potassium hydroxide (1.5 g) and mannitol (1.5 g in 15 ml of water). The product was isolated with the customary washing and drying procedures. The crude product was chromatographed to afford: (i) (22R,23R,24S)-2α,3α,22,-23-*tetrahydroxy*-5α-*ergostan*-6-*one* (1) (20 mg), m.p. 252—255 °C (from ethyl acetate); $[\alpha]_D^{20}$ −4°; $\nu_{max.}$ 1 695 cm^{-1}; δ 0.69

(3 H, s), 0.76 (3 H, s), 2.70 (1 H, m), 3.56 (1 H, d), 3.72 (1 H, d), 3.78 (1 H, m), and 4.05 (1 H, m); *m/z* 464 (M^+) (Found: C, 72.5; H, 10.35. $C_{28}H_{48}O_5$ requires C, 72.44; H, 10.41%). The tetra-acetate of (1) melted at 215—217 °C; $[\alpha]_D^{21}$ 7.1° ($CHCl_3$) (lit.,[3d] 215—217 °C; $[\alpha]_D^{24.5}$ 6.81°); (ii) (22S,23S,24S)-2α,3α,22,23-*tetrahydroxy*-5α-*ergostan*-6-*one* (13) (130 mg), m.p. 209—210 °C (from ethyl acetate); $[\alpha]_D^{20}$ 0°; $\nu_{max.}$ 1 695 cm^{-1}; δ 0.69 (3 H, s), 0.76 (3 H, s), 2.70 (1 H, m), 3.56 (1 H, d), 3.72 (1 H, d), 3.78 (1 H, m), and 4.05 (1 H, m); *m/z* 464 (M^+) (Found: C, 72.5; H, 10.35. $C_{28}H_{48}O_5$ requires C, 72.44; H, 10.41%). The tetra-acetate of (13) melted at 107—110 °C; (lit.,[3d] 108—110 °C); $[\alpha]_D^{20}$ 2° ($CHCl_3$).

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