Synthesis of Brassinolide, a Steroidal Lactone with Plant-growth Promoting Activity

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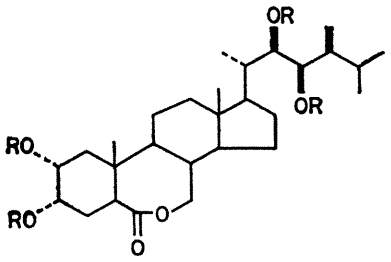
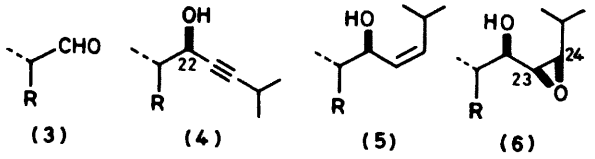
Summary Brassinolide, a plant-growth promoter isolated from rape pollen, was stereoselectively synthesized from dinorcholenic acid

BRASSINOLIDE (**1**), a plant-growth promoter isolated from rape pollen, is the first natural steroid containing a B-ring lactone and a 22*R*-oxygen function in the side chain [1] We describe the first synthesis of (**1**) from the commercially available dinorcholenic acid [2]

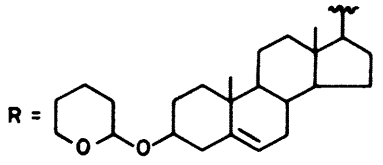


(1), R = H
(2), R = Ac

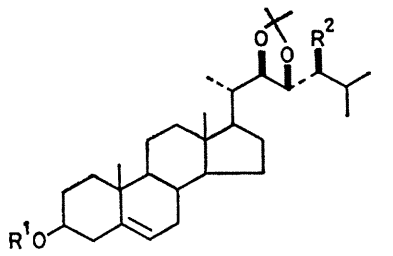
The 22-aldehyde (**3**)[3] derived from the acid was treated with 3-methylbut-1-ynyl-lithium in tetrahydrofuran (THF) at −78 °C to give a 1 : 1 epimeric mixture of the 22-alcohol, from which the more polar 22*R*-isomer (**4**), m.p. 154—155 °C was isolated by single crystallization in 38% yield.[4] Reduction of (**4**) with Lindlar catalyst afforded the *cis*-allylic alcohol (**5**), m.p. 156—158 °C, in 97% yield. Oxidation of (**5**) with t-butyl hydroperoxide–oxovanadium acetylacetonate in benzene at 20 °C for 2·5 h occurred stereospecifically, probably *via* the preferred transition state proposed by Sharpless,[5] to yield the (23*R*,24*R*)-epoxide (**6**) [m.p. 190—191 °C, δ 3·1 (1H, dd, *J* 4 and 6·5 Hz, 23-H), 85%]. Attempts to introduce a methyl-group at the C-24 of (**6**) using various reagents, *e.g.* Me_2CuLi and MeMgBr–CuI, were unsuccessful. The acetate† derived from (**6**) was subjected to hydrocyanation[6] (excess of HCN–Et_3Al in THF at 0 °C for 3 h and then at 20 °C overnight) followed



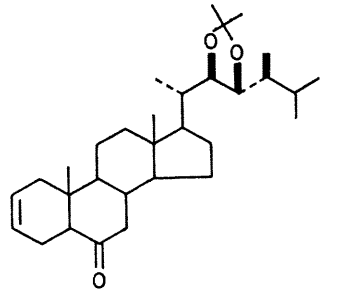
(3) (4) (5) (6)



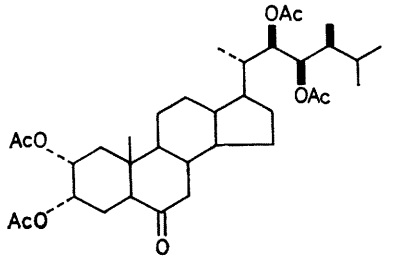
by saponification, acetonide formation, and deprotection, to give the nitrile (**7**), m.p. 222—224 °C, in 56% yield. The ^{13}C n.m.r. spectrum of (**7**) indicated that it is a single compound[7] and its stereochemistry at C-24 was assigned from the established[6] mode (*trans*) of the epoxide opening on hydrocyanation. Reduction of (**7**) with di-isobutylaluminium hydride in THF–hexane and hydrolysis gave the aldehyde (**8**), m.p. 168—171 °C, in 65% yield. Transformation of the formyl- into the methyl-group was accomplished in a 77% overall yield by the sequence: acetylation, $NaBH_4$ reduction, methanesulphonation, iodide substitution



(7), R^1=H ; R^2=CN
(8), R^1=H ; R^2=CHO
(9), R^1=Ac; R^2=Me
(10), R^1=Ms; R^2=Me



(11)



(12)

and Bu^n_3SnH reduction, to give the acetate (**9**), m.p. 148—151 °C.

Hydroboration (excess of BH_3–THF complex in THF at 20 °C for 5 h) of the corresponding methanesulphonate (**10**) and alkaline H_2O_2 oxidation, followed by oxidation with pyridinium chlorochromate and treatment with LiBr in dimethylformamide at reflux, gave the ketone (**11**), m.p. 235—237 °C, in 65% yield. Treatment of (**11**) with *N*-methylmorpholine *N*-oxide in the presence of a catalytic amount of OsO_4[8] afforded, after deprotection (70% acetic acid, 60 °C) followed by acetylation, the tetra-acetate (**12**), m.p. 215—217 °C, in 80% yield. Baeyer–Villiger oxidation of (**12**) was carried out with an excess of trifluoroperacetic acid in CH_2Cl_2 in the presence of Na_2HPO_4 at 0 °C for 3 h to give the lactone (**2**), δ 3·0 (dd, *J* 10 and 6 Hz, 5-H) and 4·1 (m, 7-H), in 80% yield.[9] Saponification of (**2**) followed by

† Similar hydrocyanation of (**5**) occurred non-regioselectively to give a mixture of the 24-cyano-22,23-diol and the 23-cyano-22,24-diol.

‡ The reported m.p.s. of natural brassinolide and synthetic 24-epi-brassinolide are 274—275 (ref. 1) and 256—258 °C (ref. 2a), respectively.

acidification by dilute HCl produced, in 68% yield, brassinolide (**1**), m.p. 273—278 °C (decomp.),‡ $[\alpha]_D^{27}$ +16°. The i.r., electron-impact mass, and ^{13}C n.m.r. spectra of (**1**) are in good agreement with those of natural brassinolide.

The synthetic (**1**) showed a high biological activity in the elongation test using wheat coleoptile.

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[1] M. D. Grove, G. F. Spencer, W. K. Rohwedder, N. Mandava, J. F. Worley, J. D. Warthen, Jr., G. L. Steffens, J. L. Flippen-Anderson, and J. Cook, Jr., *Nature (London)*, 1979, **281**, 216.

[2] Non-stereoselective syntheses of 24-epi-brassinolide from ergosterol and of a stereoisomeric mixture of 24-homo-brassinolide from stigmasterol were recently described: (a) M. J. Thompson, N. Mandava, J. L. Flippen-Anderson, J. F. Worley, S. R. Dutky, W. E. Robbins, and W. Lusby, *J. Org. Chem.*, 1979, **44**, 5002; (b) K. Mori, *Agric. Biol. Chem.*, 1980, **44**, 1211.

[3] J. A. Edwards, J. S. Mills, J. Sundeen, and J. H. Freed, *J. Am. Chem. Soc.*, 1969, **91**, 1248.

[4] The stereochemistry of (**4**) was confirmed by its conversion (H_2, 5% Pd–C in AcOEt) into the known (22*S*)-22-hydroxycholesterol (E. P. Burrows, G. M. Hornby, and E. Caspi, *J. Org. Chem.*, 1969, **34**, 103). The 22*S* isomer of (**4**) secured from the mother liquor was transformed into (**4**) in 65% yield by reaction of the corresponding methanesulphonate with KO_2; *cf.* M. Ishiguro, H. Saito, A. Sakamoto, and N. Ikekawa, *Chem. Pharm. Bull.*, 1978, **26**, 3715.

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[7] ^{13}C N.m.r. spectroscopy is effective for distinguishing the C-24-stereoisomers of C-24-substituted steroids: S. Seo, Y. Tomita, and K. Tori, *J. Chem. Soc., Chem. Commun.*, 1978, 319; N. Koizumi, Y. Fujimoto, T. Takeshita, and N. Ikekawa, *Chem. Pharm. Bull.*, 1979, **27**, 38.

[8] V. Rheenen, R. C. Kelly, and D. Y. Cha, *Tetrahedron Lett.*, 1976, 1973.

[9] Separate experiments indicated that the ratio of the 6-oxaketone to the 7-oxaketone from the steroidal 3β-acetoxy-6-ketone on Baeyer–Villiger oxidation was 1:1, while that from the 2α,3α-diacetoxy-6-ketone was 1:10; *cf.* R. C. Cookson, R. P. Gandhi, and R. M Southam, *J. Chem. Soc. C*, 1968, 2494.