Corporation. We thank Alexander Vasilakis for his assistance in preparing some of the starting materials.

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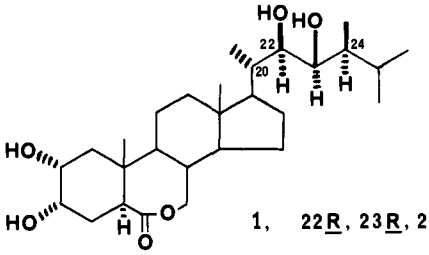
Stereoselective Synthesis of Brassinolide: A Plant Growth Promoting Steroidal Lactone

Sir:

The structure and stereochemistry of brassinolide (**1**) were determined[1] recently by X-ray crystallography, after isolation of



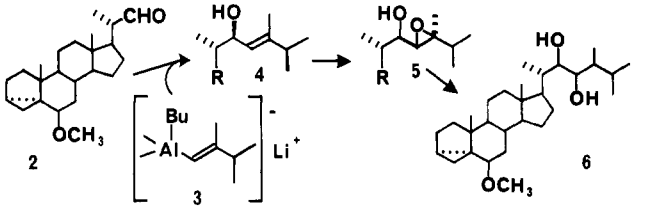
1, 22R, 23R, 24S

4 mg from 40 kg of bee-collected pollen of *Brassica napus* L. (rape). Brassinolide promotes cell division, cell elongation, and plant growth. For high activity, both the B-ring lactone and the configuration at C-24 were found[2] to be important. The novel biological activity and the scarcity of this natural product stimulated our work in which we report the first synthesis of brassinolide.

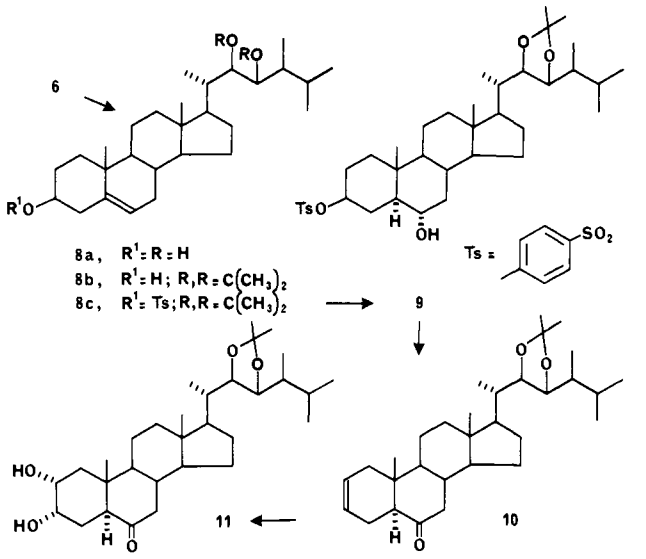
Our plan for construction of the dihydroxy side chain generates four contiguous asymmetric centers by using stigmasterol's chiral C-20 to generate asymmetry first at C-22, which in turn controls the stereochemistry of C-23 and C-24 during hydroxyl-directed epoxidation of **4**. Inversion of configuration at C-24 upon anti-Markovnikov reduction of epoxide **5** completes the three-step synthesis of the side chain. An alternative direct hydroboration-oxidation of the *Z* isomer of **4** to glycol **6** was expected to offer less stereochemical control. The choice of first elaborating the side chain and then the nucleus requires only one protecting group in the 11 steps to brassinolide from the 3,5-cyclo steroidal aldehyde **2** (prepared easily[3-5] from stigmasterol).

Stereoselective alkylation of aldehyde **2** with lithium butyldimethyl (*E*)-2,3-dimethylbutenylalanate[6] (**3**) gave in 46% yield, after chromatography on silica gel, the major 22*S*-allylic alcohol

Scheme I



Scheme II



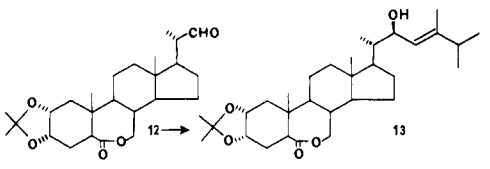
4[7,8] [mp 127-129 °C; NMR ($CDCl_3$) δ 1.60 (d, J = 1.5 Hz, 3 H, H-28); monoacetate,[7,8] mp 113-114 °C] (Scheme I). In addition to traces of aldehyde **2**, the less polar 22*R* isomer of **4** was separated as a glass in 8% yield, indicating ca. 85:15 stereoselectivity, which compares favorably with alkenyllithium alkylations[5] of **2**.

Hydroxyl-directed epoxidation of **4** with *m*-chloroperbenzoic acid (CH_2Cl_2, 22 °C, 12 h) showed 95:5 stereoselectivity whereas *t*-BuOOH/$VO(acac)_2$ in toluene (0 °C, 3.5 h) gave an 85:15 ratio of the same epoxides, indicating a threo-selective conversion similar to those reported[9] for 2-methylpent-2-en-4-ol. Recrystallization gave pure epoxide **5**[7,8] [mp 98-99 °C; NMR ($CDCl_3$) δ 1.22 (s, 3 H, H-28), 2.77 (d, J = 7 Hz, 1 H, H-23), 3.54 (br d, J = 7 Hz, 1 H, H-22)] whose NMR coupling constant J = 7 Hz, for H-22 to H-23, and chemical shift of H-22 are consistent with those reported but are not definitive for threo epoxides.[10]

Completion of the side-chain synthesis by anti-Markovnikov reduction of **5** with inversion[11] at C-24 ($LiBH_4$, BH_3·THF; 50 °C, 20 h) showed 3:1 regioselectivity for formation of the vicinal glycol **6**[7] [mp 70-73 °C; ^{13}C NMR ($CDCl_3$) δ 10.3, 11.92, 12.14, 20.70, 20.90 (CH_3), 73.39, 74.80 (C-O)]. The minor 1,3-diol **7**[7,8] [mp 159.5-160 °C; NMR δ 1.19 (s, 3 H, H-28)] was separated initially by chromatography but does not form an acetonide, which simplified purification of crude reduction product **6** + **7**.

At this point, the close similarity of chemical shifts in the ^{13}C NMR spectrum of **6** with the relevant seven shifts of those reported[1] for brassinolide strongly supported[12] the assumed 22*R*,23*R*,24*S* configurations of **6**. Proof of the absence of racemization at C-20 came from $NaIO_4$ cleavage of diol **6** to give aldehyde **2**.

(1) Grove, M. D.; Spencer, G. F.; Rohwedder, W. K.; Mandava, N.; Worley, J. F.; Warthen, J. D., Jr.; Steffens, G. L.; Flippen-Anderson, J. L.; Cook, J. C. *Nature (London)* **1979**, *281*, 216-217.
(2) Thompson, M. J.; Mandava, N.; Flippen-Anderson, J.; Worley, J. F.; Dutky, S. R.; Robbins, W. E.; Lusby, W. *J. Org. Chem.* **1979**, *44*, 5002-5004.
(3) Hutchins, R. F. N.; Thompson, M. J.; Svoboda, J. A. *Steroids* **1970**, *15*, 113-120.
(4) Salmond, W. G.; Sobala, M. C. *Tetrahedron Lett.* **1977**, 1695-1698.
(5) Wiersig, J. R.; Waespe-Sarcevic, N.; Djerassi, C. *J. Org. Chem.* **1979**, *44*, 3374-3382.
(6) Prepared from 3-methyl-1-butyne and trimethylaluminum with dicyclopentadienylzirconium dichloride catalysis, then butyllithium in hexane with the following procedure: Okukuda, N.; Negishi, E. *Tetrahedron Lett.* **1978**, 2357-2360. See also: VanHorn, D. E.; Negishi, E. *J. Am. Chem. Soc.* **1978**, *100*, 2252-2254. The alkylation reaction at 0 °C and then 22 °C for 14 h was initially 0.2 M in lithium reagent (1.5 equiv) in 30% ether/hexane. Similar alkenylations of aldehydes to form disubstituted alkenyl alcohols in 30-50% yield were reported by Newman, H. *Tetrahedron Lett.* **1971**, 4571-4572. The intermediate dimethyl[(*E*)-2,3-dimethylbutenyl]alane also alkylates aldehyde **12** to give **13** selectively in our alternate approach to the synthesis of **1**, which is in progress.



(7) This compound showed IR, NMR, electron impact, and/or chemical ionization mass spectral data fully compatible with the indicated structure.
(8) Elemental analyses for C, H within 0.3% of theory were obtained for this compound.
(9) Rossiter, B. E.; Verhoeven, T. R.; Sharpless, K. B. *Tetrahedron Lett.* **1979**, 4733-4736.
(10) Mihelich, E. D. *Tetrahedron Lett.* **1979**, 4729-4732.
(11) Brown, H. C.; Yoon, N. M. *J. Am. Chem. Soc.* **1968**, *90*, 2686-2688.
(12) Letourneaux, Y.; Khuong-Huu, Q.; Gut, M.; Lukacs, G. *J. Org. Chem.* **1975**, *40*, 1674-1675.

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The nucleus was then developed from **6** by acid-catalyzed regeneration[5] of the 3β-hydroxy-5-ene in **8a**[7] (mp 205–208 °C) (Scheme II) protected as the acetonide **8b**[7,8,13] (mp 130–131 °C) to allow tosylation at C-3 to form **8c**[7] (mp 69–70 °C). Oxygen was introduced at C-6 by hydroboration–oxidation (BH_3·THF, O °C, 1.5 h then 16 h at 22 °C) of **8c** to give **9**,[7] which underwent smooth elimination with Li_2CO_3 in dry dimethylacetamide (150 °C, 15 min) followed by Jones oxidation to give the 6-ketone **10**[7,8] (mp 228–229 °C) after silica gel chromatography.

Stereospecfic α-face hydroxylation (OsO_4, C_5H_5N; O °C, 3 h) of **10** gave the 2α,3α-diol **11**[7] (mp 216–218 °C) which was simultaneously deprotected and Bayer–Villiger oxidized in the final step. Thus, addition of **11** in CH_2Cl_2 to 3 equiv of ice-cold 0.6 M CF_3CO_3H[14] in moist CH_2Cl_2/CF_3CO_2H leads cleanly in 1 h at 22 °C to brassinolide **1**[7] in 74% yield[15,16] after recrystallization from aqueous methanol [mp 273–274 °C (lit.[1] mp 274–275 °C)]. The synthetic brassinolide in chemical ionization mass spectrometry (CH_4 reagent gas) showed ions at *m/e* 481 (100, M + 1), with four losses of H_2O at 463 (89), 445 (46), 427 (33), and 409 (21), and C-22–23 cleavage at 379 (36), 361 (45,379–H_2O).

The identity of synthetic with natural brassinolide was shown by ^{13}C NMR spectral coincidence (within 0.07 ppm) of all the lines observed in the CD_2Cl_2–CD_3OD (9:1) solution with those cited[1] for brassinolide. Biological activity of brassinolide is not diagnostic for side-chain stereochemistry since two synthetic stereoisomers (22*S*,23*S*,24*R* and 22*R*,23*R*,24*R*) were found[2] to be less potent but quite active at 10 ng/plant in pinto bean assays.[1] Extensive biological investigation of natural brassinolide was hindered by low availability, but we anticipate that more interesting studies may now be made possible by this work. Our beginning studies of its biological properties will be reported subsequently.

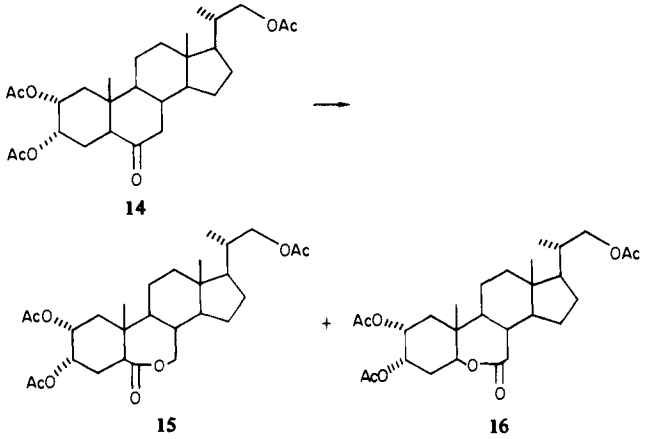
Acknowledgment. We thank Drs. M. Maddox (Syntex), J. Shoolery (Varian) and L. J. Durham (Stanford) for invaluable NMR spectroscopy data and G. Jamieson for mass spectroscopy data.

(13) Vicinal coupling of J = 8.5 Hz for H-22 to H-23 observed for **8b** is compatible with both threo and erythro relationships: Gregson, M.; Ollis, W. D.; Redman, B. T.; Sutherland, I. O.; Dietrichs, H. H. *Chem. Commun.* **1968**, 1394–1395.

(14) Prepared by adding $(CF_3CO)_2O$ (6.74 mL) to 30% aqueous H_2O_2 (1 g) in CH_2Cl_2 (7.4 mL) at 0 °C; dilution to 0.2 M in peracid results on addition of **11** in CH_2Cl_2. Oxidation[2] by *m*-$ClC_6H_4CO_3H$ is over 1000 times slower!

(15) Mother liquors contained some trifluoroacetates, recoverable as **1** by aqueous K_2CO_3 hydrolysis, with CH_3CO_2H for relactonization.

(16) Migration of C-5 leads to an isomeric 6-oxa-*B*-homo-7-one as the minor product in the "anomalous" Bayer–Villiger oxidation of 5α-6-keto steroids (ref 2 and 10 therein). We found by capillary GLC analysis that lactones **15** and **16** are produced in an 88:12 ratio when the CF_3CO_3H reagent[14] oxidizes **14**, in an alternate approach to the synthesis of brassinolide. The minor isomer from oxidation of **11** was not characterized.



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New Manganese(III)-Containing Acid Phosphatase. Evidence for an Intense Charge-Transfer Band and Tyrosine Phenolate Coordination

Sir:

Only a few enzymes, such as pyruvate carboxylase,[1] superoxide dismutase (SOD),[2] and diamine oxidase,[3] contain tightly bound manganese. Studies of the coordination chemistry of biological manganese have been limited and directed primarily to Mn(II) species.[4] However, recent magnetic susceptibility and electron spin resonance (ESR) experiments demonstrated that the metal bound to Mn–SOD of *E. coli* is trivalent.[5] We have isolated a Mn(III)-containing acid phosphatase and characterized the unique metal chromophore.

The purification and crystal preparation of the Mn–enzyme complex from the tuber of the sweet potato (Kintoki) and the enzymatic properties of the native enzyme will be fully described elsewhere. The manganese ion present at one atom per enzyme molecule (M_w 110 000) plays an essential role in the catalytic reaction of hydrolysis of phosphomonoesters and nucleotide phosphates.[6] This stable metalloenzyme is violet in color with an intense absorption band at 515 nm (ε 2460) attributed to the Mn ion directly coordinated with some amino acid residues. The extinction coefficient of the enzyme is significantly larger than that of the *E. coli* Mn–SOD complex [λ_{max} 473 nm (ε 400)].[2a] The ratio of Δε to ε is 2.1×10^{-4} for the characteristic visible band. The 550-nm extremum band (Δε −0.53) in the circular dichroism (CD) spectrum was used to determine Δε/ε. In a rough approximation, $\gamma = |\Delta\epsilon/\epsilon|$ can be utilized to estimate Kuhn's anisotropic factor, where Δε and ε are the CD($\epsilon_L - \epsilon_R$) and optical absorption in terms of extinction coefficients, respectively.[7] The ratio is typically $\geq 10^{-2}$ for magnetically allowed and electrically forbidden transitions of the d-d type. Therefore, the intense 515-nm band is assigned to an electrically allowed charge-transfer band from the ligand to the metal, which is expected for Mn(III) rather than Mn(II).[8,9]

Figure 1 shows the ESR spectra of the native (A) and denaturated (B) enzymes. The X-band ESR spectra were obtained at 293 K with a JES-FE-3X spectrometer. ESR signals were not obtained with the native violet enzyme. In contrast, the acid- and heat-treated colorless enzyme showed typical six-line ESR patterns due to the aquated Mn(II) ion (^{55}Mn, $I = {}^5/_2$) around $g = 2$. Similar ESR behavior has been observed in the Mn–SOD complex.[2a] Fee et al. reported that the absence of an observable ESR signal in the Mn–SOD complex is quite characteristic of a Mn(III) ($S = 2$) integral spin system with zero-field splitting of 1–2 cm^{-1}.[5] These visible and ESR results strongly indicate that the Mn valence state of the native acid phosphatase is trivalent, Mn(III).[10]

Figure 2 shows the resonance Raman spectrum of the native Mn-containing acid phosphatase. The present spectrum was

(1) Scrutton, M. C.; Utter, M. F.; Mildvan, A. S. *J. Biol. Chem.* **1966**, *241*, 3480–3487.

(2) (a) Keele, B. B.; McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1970**, *245*, 6176–6181. (b) Weissiger, R. A.; Fridovich, I. *Ibid.* **1973**, *248*, 3582–3592.

(3) Crabbe, M. J. C.; Waight, R. D.; Bardsley, W. G.; Barker, R. W.; Kelly, I. O.; Knowles, P. F. *Biochem. J.* **1976**, *155*, 679–687.

(4) Lawrence, G. D.; Sawyer, D. T. *Coord. Chem. Rev.* **1978**, *27*, 173–193.

(5) Fee, J. A.; Shapiro, E. R.; Moss, T. H. *J. Biol. Chem.* **1976**, *251*, 6157–6159.

(6) The Mn-removed apoenzyme was catalytically inactive. In addition, the enzyme activity was reduced in parallel with decrease in the 515-nm absorbance.

(7) Gillard, R. D. In "Physical Methods in Advanced Inorganic Chemistry"; Hill, H. A. O., Day, P., Ed.; Interscience: London, 1968; pp 167–213.

(8) (a) Dunn, T. M. In "Modern Coordination Chemistry"; Lewis, J.; Wilkins, R. G., Ed.; Interscience: New York, 1960; pp 229–300. (b) Dingle, R. *Acta Chem. Scand.* **1966**, *20*, 33–44.

(9) In the ferric enterobactin and tris(catecholate) complexes, it is known that a broad absorption band centered at ~500 nm is due to phenolate bound to Fe(III) and is assigned to a phenolate → Fe(III) charge-transfer transition: Gaber, B. P.; Miskowski, V.; Spiro, T. G. *J. Am. Chem. Soc.* **1974**, *96*, 6868–6873.

(10) An alternative interpretation for the loss of the ESR signal is that Mn(II) is tightly bound to protein and thereby immobilizes within its ligand field. In such a case, however, the intensely visible band would not appear.

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