acid–CH_2Cl_2, 20 mL; (19) 2 × 3 nm CH_2Cl_2, 20 mL; (20) 2 × 3 nm EtOH, 20 mL; (21) 4 × 3 nm CH_2Cl_2, 20 mL; (22) 3 × 3 nm 5% DIEA–CH_2Cl_2, 20 mL; (23) 4 × 3 nm CH_2Cl_2, 20 mL. Washes in steps 22 and 23 were collected and their absorbance was measured at 362 nm.

Registry No. Ia, 538-75-0; Ib, 693-13-0; Ic, 21002-18-6; Id, 38463-75-1; Ie, 63796-18-9; IIa, 2387-23-7; IIb, 4128-37-4; IIc, 6957-05-7; IId, 71819-34-6; IIe, 61843-91-2; ethyl isocyanate, 109-90-0; ispropyl isocyanate, 1795-48-8; benzenamine, 62-53-3; benzylamine, 100-46-9.

Communications

Synthesis of Brassino Steroids: New Plant-Growth-Promoting Steroids

Summary: We have synthesized two highly physiologically active brassino steroids, structural isomers of brassinolide (2α,3α,22(*R*),23(*R*)-tetrahydroxy-24(*S*)-methyl-*B*-homo-7-oxa-5α-cholestan-6-one), the recently characterized plant growth promoting steroid isolated from rape pollen.

Sir: Lipoidal substances that have growth-promoting effects on plants have been extracted from the pollen[1] of rape (*Brassica napus* L.). One of these plant growth promoters, brassinolide, has been identified by physical methods, including X-ray analysis,[2] which showed it to be 2α,3α,22(*R*),23(*R*)-tetrahydroxy-24(*S*)-methyl-*B*-homo-7-oxa-5α-cholestan-6-one (**1**). We now wish to report the first synthesis of two 22,23 isomeric brassino steroids and a nonlactonic steroid with plant-growth-promoting activity.

Solvolysis of ergosterol tosylate (**2**) according to reported methods[3] gave i-ergosterol (**3**) (Scheme I). Oxidation of **3** to **4** with chromic acid in pyridine[4,5] followed by the reduction of **4** with lithium and liquid ammonia yielded compound **5**. Acid rearrangement of **5** by refluxing it for 2 h in acetic acid–5 N sulfuric acid[5] (20 mL/5 mL per gram of the ketone[6]) followed by saponification of the resulting acetate gave 3β-hydroxy-24β-methyl-5α-cholest-22-en-6-one (**6a**). Compound **6a**, mp 186–187 °C, $[\alpha]^{25}_D$ −35°, was obtained from ergosterol without purification of any intermediates in an overall 33% purified yield via the described sequence of reactions.

The detosylation of **6b** in dimethylformamide, which contained 10% each of lithium bromide and **6b** at reflux temperature for 45 min, gave **7** in 70% purified yield, mp 123–124 °C, $[\alpha]^{25}_D$ +3°. Treatment of **7** for 3 days at room temperature in dry benzene (60 mL/g) that contained a trace of pyridine and 2 molar equiv of osmium tetroxide gave nearly quantitative yield on reductive cleavage of the osmate ester 1:1 mixture[7] of the tetrahydroxy ketones **8a** (R_f 0.53) and **9a** (R_f 0.45)[8] with the expected 2α,3α-*cis*-diol orientation.[9] The tetrahydroxy ketones were separated by column chromatography over Woelm neutral alumina (activity grade III) in a gradient-type elution system progressing from chloroform–benzene (90:10) through chloroform–methanol (1:1). The tetrahydroxy ketone (**8a**), with an R_f value of 0.53, was recrystallized from ethyl acetate, mp 182–183 °C, $[\alpha]^{25}_D$ −2°. A similar recrystallization of the component with an R_f value of 0.45 gave **9a**, mp 241–242 °C, $[\alpha]^{25}_D$ 0°.

A Baeyer–Villiger oxidation[10] of the tetraacetates **8b** and **9b** in chloroform with *m*-chloroperbenzoic acid for 2 weeks[11] at room temperature gave predominantly the crude tetraacetoxy-7-oxa ketones **10a** and **11a**, respectively. Both lactones also contained a small quantity of the respective isomeric 6-oxa ketone. The lactones **10a** and **11a** were purified by column chromatography over Unisil[12] by initially eluting the columns with benzene–chloroform (90:10) and then with increasing percentages of chloroform in benzene. Saponification of **10a** with 4% potassium carbonate in refluxing 70% aqueous methanol for 4 h, followed by acidification with dilute hydrochloric acid solution, and subsequent recrystallization of the precipitate from ethyl acetate gave (in 25% overall purified yield from **7**) 2α,3α,22β,23β-tetrahydroxy-24β-methyl-*B*-homo-7-oxa-5α-cholestan-6-one (**10b**): mp 194–195 °C; $[\alpha]^{25}_D$ +31°; NMR (C_5D_5N) δ 4.1 (2, d, 7a-H, J = 4 Hz), 0.63 (3, s, 18-H), 1.03 (3, s, 19-H); EI–MS showed no M^+ at m/e 480, the first observable peak at m/e 462 ($M^+ - H_2O$, <1), and other ions at m/e 447 (3), 409 (3), 380 ($M^+ - 100$, 27) results from cleavage of 22,23 carbon bond, 362 (20), 350 (24), 343 (15), 333 (14), 319 (10), 303 (12), 285 (11), 208 (14), 189 (19), 177 (25), 107 (52), 81 (77), 71 (58), 43 (100); CI–MS (isobutane) showed ions at m/e 481 (M + 1), 463 (M + 1 − H_2O), 445 (M + 1 2 H_2O), 427 (M + 1 − 3H_2O), 409 (M + 1 − 4H_2O), 379, 361, 349, 321, 303.

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(5) G. H. R. Summers, *J. Chem. Soc.*, 4489 (1958).

(6) Since the intermediates **2** through **5** were not purified, the quantity of **5** present was based on the calculated weight of **4** by UV analysis.

(7) Hydroxylation of **6b** via the osmate ester also gave a 1:1 mixture of compounds from which compounds **8a** and **9a** were also obtained through further reactions of hydroxylated products of **6b**. Thus the mixture results from hydroxylation of the side chain.

(8) From silica gel TLC plate developed twice in the solvent system of chloroform–ethanol (7:1).

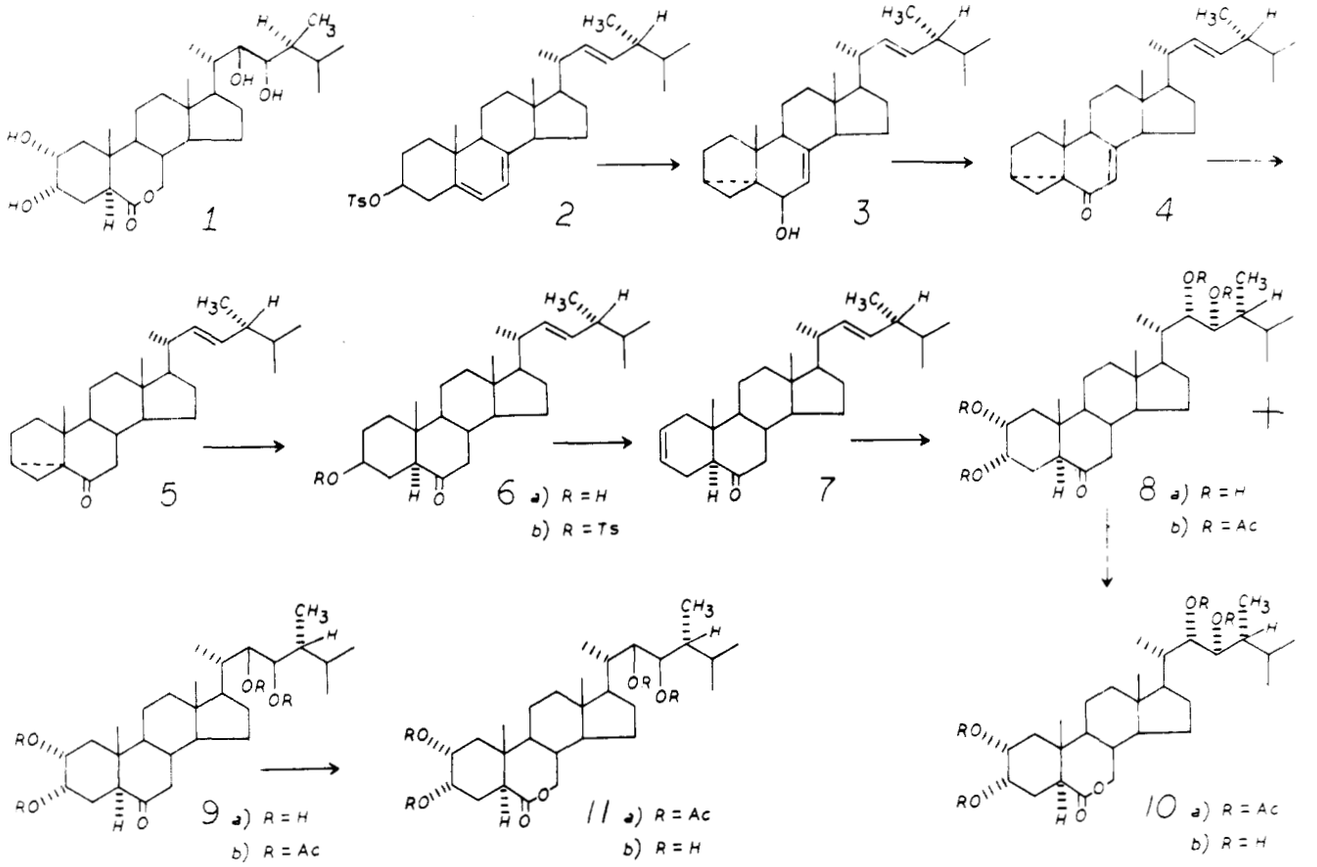
(9) L. Fieser and M. Fieser, "Steroids", Reinhold, New York, 1959, p 274.

(10) (a) R. C. Cookson, R. P. Gandhi, and R. M. Southam, *J. Chem. Soc. C*, 2494 (1968); (b) M. S. Ahmad, G. Moinuddin, and I. A. Khan, *J. Org. Chem.*, **43**, 163 (1978).

(11) The progress of the reaction was monitored by TLC.

(12) (a) With Unisil (silicic acid) as an adsorbent there is no loss of lactones during chromatography though removal of contaminants was less effective than with Woelm neutral alumina (activity Grade II). (b) Mention of a company name or proprietary product in this paper does not constitute an endorsement of the product by the U.S. Department of Agriculture.

Scheme I



Saponification of **11a** followed by recrystallization of the tetrahydroxy lactone **11b** from ethyl acetate gave, in 20% overall purified yield from **7**, 2α,3α,22α,23α-tetrahydroxy-24β-methyl-*B*-homo-7-oxa-5α-cholestan-6-one (**11b**): mp 256–258 °C; $[\alpha]^{25}_{D}$ +30°; NMR (C_5D_5N) δ 4.1 (2, d, 7a-H, J = 4 Hz), 0.70 (3, s, 18-H), 1.03 (3, s, 19-H). The CI–MS and EI–MS of **11b** showed a fragmentation pattern similar to that of **10b** and brassinolide (**1**) and were consistent with the expected cleavage of the dihydroxylated side chain.

Although the method of synthesis allowed us to establish the basic structures of **10b** and **11b**, X-ray analyses were required for the rapid determination of the orientation of the hydroxyl groups at C-22 and C-23. Compound **10b** crystallized in the orthorhombic space group $P2_12_12_1$ with a = 11.399, b = 36.018, and c = 6.395 Å and one molecule in the asymmetric unit. The structure was established by application of the symbolic addition procedure for noncentrosymmetric crystals[13] followed by tangent formula refinement and expansion.[14] Compound **11b**, like brassinolide,[2] crystallized in the monoclinic space group $P2_1$ with a = 9.880, b = 7.521, and c = 18.365 Å and β = 94.7°. These values for **11b** compare with those of brassinolide.[2]

The X-ray analysis showed that the hydroxyl groups at C-22 and C-23 in **10b** were projected to the rear of the side chain. Since oxidation of an olefinic bond with osmium tetroxide gives only a *cis*-glycol, the hydroxyl groups at C-22 and C-23 of the epimer **11b** are directed toward the front. Thus, the lactones **10b** and **11b** have the assigned designations,[15] and it follows that compounds **8a** and **9a** have the 22β,23β- and 22α,23α-hydroxy orientation, respectively. The X-ray analyses also indicate that the steroid nuclei of **10b**, **11b**, and brassinolide are identical and that brassinolide (**1**) differs from **10b** at C-22, C-23, and C-24; on the other hand, **1** differs from **11b** only in the orientation of the methyl group at C-24.

In the bean second internode bioassay, compounds **9a**, **10b**, and **11b** showed brassin activity, a unique biological response of cell elongation and cell division that results in elongation, curvature, swelling, and finally splitting of the treated internodes.[1,16]

This synthesis of compounds with brassin activity and the comparative X-ray analyses confirm the structure of the steroidal nucleus of brasinolide. We now have available a method for the preparation of gram quantities of material for greenhouse and field evaluation of the effects of these unique hormonal growth substances on plant growth and crop production.

Registry No. **1**, 72050-65-8; **2**, 51373-27-4; **3**, 2774-59-6; **4**, 3037-46-5; **5**, 3152-46-3; **6a**, 72050-66-9; **6b**, 72050-67-0; **7**, 72050-68-1; **8a**, 72050-69-2; **8b**, 72050-70-5; **9a**, 72050-71-6; **9b**, 72050-72-7; **10b**, 72075-01-5; **11a**, 72059-88-2; **11b**, 72075-02-6; ergosterol, 57-87-4.

Supplementary Material Available: Experimental Section describing the preparation details of **6a**, **7**, **8a**, **9a**, **10b**, and **11b**,

(13) J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).

(14) J. Karle, *Acta Crystallogr., Sect. B*, **24**, 182 (1968).

(15) In the sequence rule of nomenclature of the steroid side chain, designation of the hydroxyl and methyl groups of **10b** would be (22*S*,23*S*,24*R*) respectively, and that of **11b** would be (22*R*,23*R*,24*R*). Accordingly, in the α,β system nomenclature of the steroid side chain, designation of the hydroxyl and methyl groups of brassinolide would be 22α,23α,24α, respectively. Substituents projecting to the rear are β (dotted line) and those to the front are α (solid line); see W. R. Nes, *Adv. Lipid Res.*, **15**, 233 (1977).

(16) The ketone **9a** induced more elongation (relative to curvature and swelling) while the ketone **8a** exhibited no brassin activity. The lactones **10b** and **11b** caused marked curvature and swelling in the concentration range of 0.01–10 μg/plant. In addition, the lactones **10b** and **11b** under certain conditions (1–10-μg range) cause an internode splitting response that is a distinguishing characteristic for brassinolide at the 0.1–10-μg range.

a perspective drawing of **10b** (Figure 1), and the plant growth response of **10b** and **11b** (Figure 2) (9 pages). Ordering information is given on any current masthead page.

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Rapid Reaction of Trialkylboranes with Lithium Aluminum Hydride in the Presence of Triethylenediamine. Facile and Quantitative Synthesis of Lithium Trialkylborohydrides Including Derivatives with Exceptionally Large Steric Requirements

Summary: Addition of 1 mol equiv of lithium aluminum hydride to a diethyl ether (EE) solution of trialkylborane, in the presence of 1 mol equiv of triethylenediamine (TED) at 0 °C, results in a facile and rapid transfer of hydride to form the corresponding lithium trialkylborohydride in quantitative yield, with concurrent precipitation of aluminum hydride–triethylenediamine. The reaction is general, applicable even to the synthesis of lithium trialkylborohydrides with exceptionally large steric requirements.

Sir: Investigations in our laboratory and elsewhere have established the importance of hindered and highly hindered trialkylborohydrides for the stereoselective reduction of ketones.[1,2] The trialkylborohydrides also react with carbon monoxide to give a highly reactive intermediate which can be converted into a variety of valuable compounds.[3,4] Another application of trialkylborohydrides is their addition to styrene and its derivatives,[5] providing a convenient synthesis of Markownikoff organoboranes. The most direct way to form such borohydrides is by the direct reaction of trialkylboranes and alkali metal hydrides. Such reactions have been carried out with lithium,[6] sodium,[6] and potassium[7] hydrides. However, all of the three alkali metal hydrides fail to react satisfactorily with highly hindered trialkylboranes, such as trisiamylborane (eq 1). Yet these are reagents of exceptional promise.

$$MH + Sia_3B \xrightarrow[24\ h]{THF,\ 25\ °C} \underset{0\text{–}10\%}{MSia_3BH} \quad (1)$$

M = Li, Na, K

The lithium hydride route provides a convenient entry only to the relatively unhindered lithium trialkylborohydrides. A general synthesis of lithium trialkylborohydrides has been developed using lithium trimethoxyaluminohydride (LTMA)[8] (eq 2). However, this suffers from the difficulty of separating the product from the aluminum methoxide formed concurrently.

$$R_3B + LiAl(OCH_3)_3H \xrightarrow[15\ min]{THF,\ 25\ °C} \underset{100\%}{LiR_3BH} + Al(OCH_3)_3 \quad (2)$$

An alternative synthesis which avoids this difficulty is the reaction of *tert*-butyllithium with trialkylboranes at low temperature[2c,9] (eq 3). However, this suffers from the cost of *tert*-butyllithium for the preparation of the reagent in quantity.

$$R_3B + (CH_3)_3CLi \xrightarrow{THF,\ -78\ °C} LiR_3BH + (CH_3)_2C{=}CH_2 \quad (3)$$

More recently, lithium aluminum hydride was evaluated for its applicability in the hydride-induced carbonylation of organoboranes.[10] The subsequent discovery that other complex hydrides used in this reaction transfer alkali metal hydride to the trialkylborane[8,11,12] generated interest in a systematic investigation of the reaction of lithium aluminum hydride with representative trialkylboranes[13] as a potential route to the lithium trialkylborohydrides.

Addition of a THF solution of lithium aluminum hydride to triethylborane resulted in a rapid, moderately exothermic reaction. Examination of the clear, colorless solution by ^{11}B NMR unexpectedly revealed a triplet (δ −16.7, J = 67 Hz), instead of the anticipated doublet. Other trialkylboranes with primary alkyl groups gave similar results. Evidently, the reaction proceeds as shown in eq 4. Reaction 4 was carried out at −78 °C and the

$$LiAlH_4 + R_3B \xrightarrow[15\ min]{THF,\ 25\ °C} LiR_2BH_2 + RAlH_2 \quad (4)$$

R = Et, *n*-Bu, *i*-Bu

solution examined as rapidly as possible by ^{11}B NMR. The results established that the reaction proceeds with the initial formation of the desired trialkylborohydride (eq 5 and 6).

$$LiAlH_4 + R_3B \xrightarrow{THF,\ -78\ °C} LiR_3BH + AlH_3 \quad (5)$$

$$LiR_3BH + AlH_3 \xrightarrow[fast]{THF,\ 25\ °C} LiR_2BH_2 + RAlH_2 \quad (6)$$

This study indicated that a new synthesis of lithium trialkylborohydride might be achieved if the aluminum hydride could be trapped as soon as formed so as to avoid the fast subsequent reaction. We had recently observed that triethylenediamine (TED) rapidly and quantitatively precipitates aluminum hydride as TED·AlH_3 from both diethyl ether (EE) and THF.[14] Accordingly, the lithium aluminum hydride solution (EE) was added to an EE solution of the trialkylborane containing 1.0 equiv of TED at 0 °C. A voluminous white precipitate of TED·AlH_3

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