

SYNTHESIS OF BRASSINOLIDE, A PLANT GROWTH PROMOTING STEROIDAL LACTONE<sup>†</sup>

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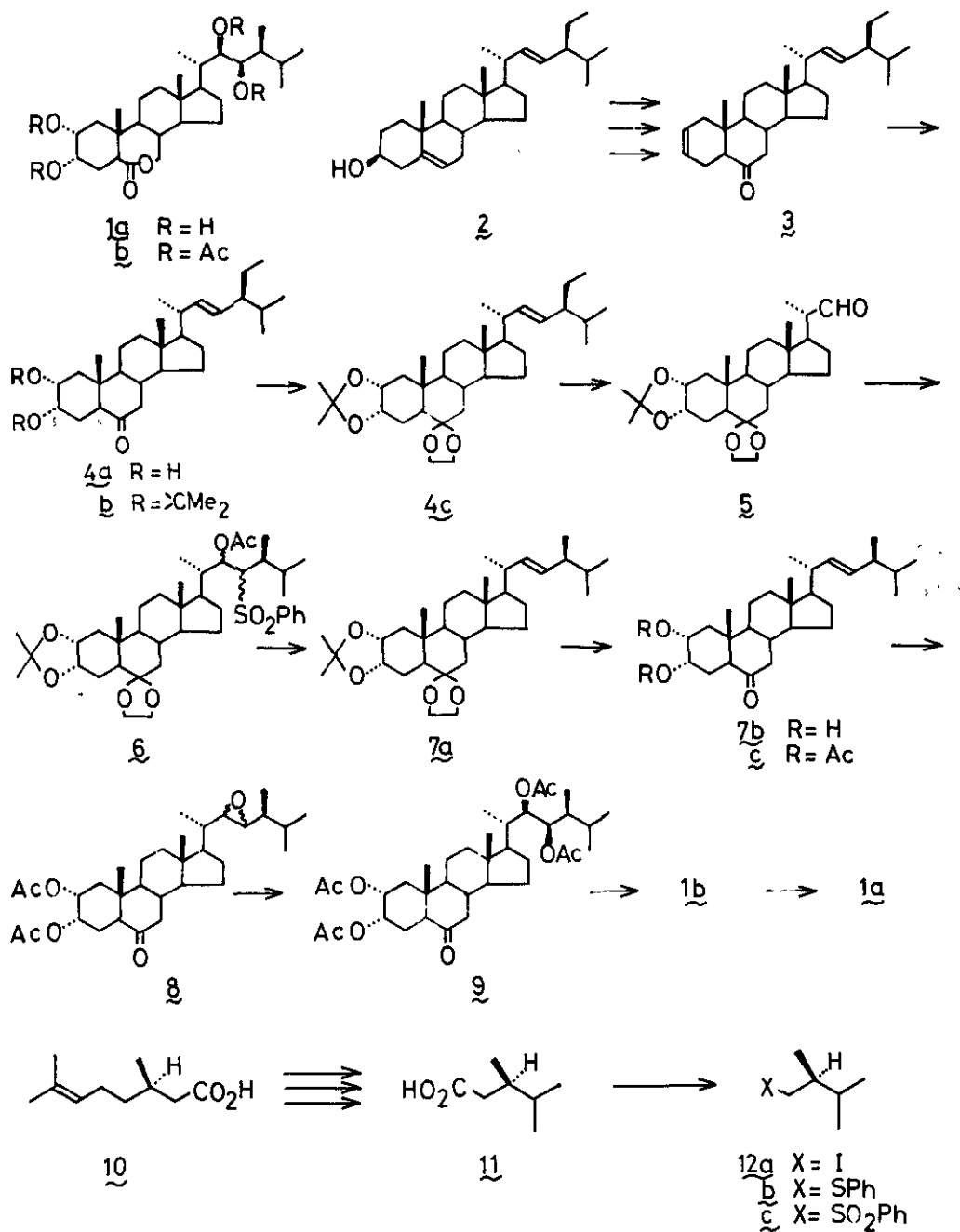
**Abstract** — Brassinolide (2 $\alpha$ , 3 $\alpha$ , 22R, 23R-tetrahydroxy-24S-methyl-B-homo-7-oxa-5 $\alpha$ -cholestan-6-one) was synthesized from stigmasterol and (R)-(+)-citronellic acid.

In 1979 a new steroid named brassinolide was isolated (4mg) from 40kg of bee-collected rape pollen (*Brassica napus* L.) and was assigned the structure 1a by X-ray analysis.<sup>1</sup> The unique structure of brassinolide coupled with its remarkable bioactivity in promoting plant growth aroused interests among synthetic chemists and two syntheses were published.<sup>2,3</sup> We report here another synthesis starting from stigmasterol 2 as an extension of our previous synthesis of (22S, 23S)-homo-brassinolide.<sup>4</sup> Similarly to the previous synthesis,<sup>4</sup> the introduction of the two hydroxyl groups on the side chain was executed by the oxidation of the double bond at C-22.

Stigmasterol 2 was converted to a dienone 3 as described previously.<sup>4</sup> This was oxidized with osmium tetroxide and N-methylmorpholine N-oxide in aqueous acetone<sup>5</sup> to give a diol 4a,<sup>6</sup> mp 235-238°;  $[\alpha]_D^{21} - 9.2^\circ$  (CHCl<sub>3</sub>), in 97.8% yield. This was converted to the corresponding acetonide 4b, mp 158-159°;  $[\alpha]_D^{24} + 21.1^\circ$  (CHCl<sub>3</sub>), in quantitative yield by treatment with 2,2-dimethoxypropane and TsOH. After protection (butanone ethylene acetal and TsOH) of the carbonyl group as an ethylene acetal, 4c was treated with ozone. Reductive work-up (dimethyl sulfide in the presence of sodium bicarbonate) of the resulting ozonide yielded an aldehyde 5, mp 118-121°;  $[\alpha]_D^{24.5} + 38.2^\circ$  (CHCl<sub>3</sub>), in 60% yield from 4b.

<sup>†</sup>Dedicated to Professor Kyosuke Tsuda on the occasion of his 75th birthday.

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Formation of the olefinic side-chain with (24S)-methyl group was accomplished by the Kocienski olefin synthesis<sup>7</sup> employing  $5$  and a phenyl sulfone  $12\text{c}$ . This sulfone  $12\text{c}$  was prepared from optically pure (R)-(+)-citronellic acid  $10$ . Conversion of  $10$  to an acid (R)- $11$ ,  $[\alpha]_{\text{D}}^{23} + 13.7^\circ$  ( $\text{CHCl}_3$ ), was carried out as described

by us in connection with the synthesis of faranalin.<sup>8,9</sup> The acid 11 afforded the desired sulfone (R)-(+)-12c,  $[\alpha]_D^{23} + 19.1^\circ$  ( $\text{CHCl}_3$ ), in 49.2% overall yield from 11 via 12a and 12b by the known method [11  $\rightarrow$  12a;  $\text{I}_2/\text{Pb}(\text{OAc})_4/\text{hv}$ , 12a  $\rightarrow$  12b:  $\text{PhSNa}$ , 12b  $\rightarrow$  12c: MCPBA].<sup>10,11</sup>

Addition of 5 to the carbanion derived from the sulfone 12c was followed by acetylation to give a  $\beta$ -acetoxy sulfone 6. Reduction of 6 with sodium-amalgam in methanol-ethyl acetate (2:1) gave an olefinic product 7a, which upon deprotection furnished a dihydroxy enone 7b, mp 223-227°;  $[\alpha]_D^{22} + 6.92^\circ$  ( $\text{CHCl}_3$ ), in 31% overall yield from 5.<sup>12</sup> The corresponding acetate 7c, mp 195-196°;  $[\alpha]_D^{24.5} + 3.1^\circ$  ( $\text{CHCl}_3$ ), was epoxidized with *m*-chloroperbenzoic acid to give an epoxide 8, mp 203-204.5°;  $[\alpha]_D^{24.5} + 16.1^\circ$  ( $\text{CHCl}_3$ ), as a stereoisomeric mixture in 62% yield. The epoxy ring in 8 was cleaved with 30% hydrobromic acid in acetic acid (room temperature, 3 hr) to give a bromo acetate by *trans*-ring-opening. Another inversion at the carbon bearing the bromine atom was effected by heating with acetic acid-water (4:1) at 100-120° for 19 hr. The product was acetylated with acetic anhydride and 4-(*N,N*-dimethylamino)pyridine in pyridine to give the desired tetraacetoxy ketone 9, mp 221-224°;  $[\alpha]_D^{24.5} + 6.81^\circ$  ( $\text{CHCl}_3$ ) [lit.<sup>3</sup> mp 215-217°; no specific rotation was reported] in 25.3% yield from 8 after chromatographic purification.<sup>13</sup> The Baeyer-Villiger oxidation of 9 with trifluoroperacetic acid in the presence of disodium hydrogen phosphate in methylene chloride yielded brassinolide tetraacetate 1b, mp 218-220°;  $[\alpha]_D^{24} + 38.96^\circ$  ( $\text{CHCl}_3$ ), in 82.9% yield after chromatographic purification.<sup>14</sup> Hydrolysis of 1b with sodium hydroxide was followed by acidification to give brassinolide 1a, mp 273-275°;  $[\alpha]_D^{24} + 41.9^\circ$  ( $\text{CHCl}_3$ -MeOH, 9:1) [lit.<sup>1</sup> mp 274-275°; lit.<sup>2</sup> mp 273-274°; lit.<sup>3</sup> mp 273-278°,  $[\alpha]_D^{27} + 16^\circ$  (no specification of the solvent)].<sup>15</sup> The <sup>13</sup>C-NMR data of our synthetic brassinolide was in very good accord with those of the natural product.<sup>1</sup>

Full details of this work as well as the synthesis of (22R, 23R)-homobraslinolide and other analogs will be reported in due course.

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11. Kocienski et al.<sup>10</sup> prepared optically impure (S)-(-)-12c,  $[\alpha]_D^{40} -12^\circ$  (CHCl<sub>3</sub>), starting from (-)-3-methylglutaric half ester obtained by resolution.
12. This olefination reaction is known to give a trans-olefin.<sup>7</sup>
13. Another product was the stereoisomeric (22S, 23S)-tetraacetoxy ketone. This was a non-crystalline gum and easily separated from the desired ketone **8** by chromatography. This stereochemical outcome was the result of double inversion at C-23 or C-24 of the epoxy ring of **8**.
14. IR (nujol) 1750 (sh.), 1740 (s), 1722 (s), 1245 (s), 1225 (s), 1050 (m), 1020 (m) cm<sup>-1</sup>; <sup>1</sup>H-NMR (400.5 MHz, CDCl<sub>3</sub>):  $\delta$  0.74 (3H, s), 0.91 (3H, d, J=6.6 Hz), 0.94 (3H, d, J=6.4 Hz), 0.96 (3H, d, J=6.4 Hz), 0.99 (3H, s), 1.01 (3H, d, J=6.8 Hz), 1.19-1.94 (m), 1.996 (3H, s), 2.001 (3H, s), 2.014 (3H, s), 2.110 (3H, s), 2.29 (1H, ddd, J=2.2, 12.4, 15.8 Hz), 3.00 (1H, dd, J=4.5, 12.1 Hz), 4.05 (1H, dd, J=9.4, 12.5 Hz), 4.13 (1H, dd, J=1.2, 12.5 Hz), 4.88 (1H, ddd, J=2.5, 4.4 and 12.5 Hz), 5.15 (1H, dd, J=0.4 and 9.3 Hz), 5.33 (1H, dd, J=1.7, 8.8 Hz), 5.37 (1H, m).
15. IR (nujol) 3450 (s), 1725 (m), 1693 (s), 1062 (s), 1022 (s), 980 (s), 965 (m) cm<sup>-1</sup>; <sup>1</sup>H-NMR (400.5 MHz, C<sub>5</sub>D<sub>5</sub>N):  $\delta$  0.72 (3H, s), 1.04 (3H, d, J=6.8 Hz), 1.05 (3H, s), 1.11 (3H, d, J=6.4 Hz), 1.14 (3H, d, J=6.8 Hz), 1.21 (3H, d, J=6.3 Hz), 2.31 (1H, dt, J=4.0, 14.5 Hz), 2.52 (1H, ddd, J=2.0, 12.0, 14.0 Hz), 3.60 (1H, dd, J=4.2, 12.0 Hz), 3.95 (1H, d, J=8.0 Hz), 3.99-4.11 (3H, m), 4.13 (1H, dd, J=0.5, 8.0 Hz), 4.43 (1H, br. s); <sup>13</sup>C-NMR (25.0 MHz; CD<sub>2</sub>Cl<sub>2</sub>-CD<sub>3</sub>OD, 9 : 1):  $\delta$  10.4, 12.0, 12.2, 15.7, 20.9, 21.1, 68.4, 68.5, 71.3, 73.7, 74.9, 178.1.

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