Intramolecular Michael–Aldol Condensation Approach to the Construction of Advanced Intermediates in the Synthesis of Forskolin

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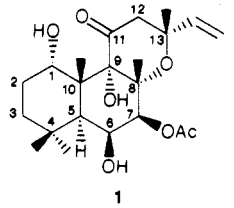
The tricyclic α,β-unsaturated ketone **6** has been elaborated from the β-keto ester **7c** by using an intramolecular Michael addition (**7c** → **10**), in tandem with an intramolecular aldol condensation (**10** → **6**). Starting from **6**, the allylic alcohols **17a** and **17b** were prepared. The stereochemical features of **17a** and **17b** were determined by analysis of their NMR spectra and further confirmed by transformation of **17b** into the known methyl ether **17c**. Through a simple synthetic sequence, **17b** was, in turn, converted to the known acetonide **19c**. Compounds **17b** and **19c** are significant intermediates for the synthesis of forskolin (**1**).

The unique structural features of the highly oxygenated labdane diterpene forskolin (**1**),[1] coupled with its remarkable biological importance,[2] make this natural product a challenging target for synthesis. Consequently, a number of synthetic approaches to **1** have been explored.[3-6] Recently, three different routes culminated successfully in the total synthesis of (±)-forskolin, all of which, interestingly enough, proceed through the intermediacy of lactone **2**.[7] This key intermediate was first synthesized



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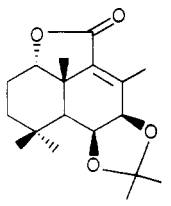

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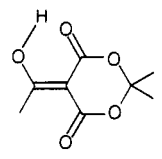
by Ziegler et al.[5c] by an intramolecular Diels–Alder strategy as shown in a simplified form in Scheme I. On the basis of the retrosynthetic analysis depicted in Scheme II, we envisioned that a shorter route to lactone **4** could be achieved by coupling an intramolecular Michael addition with an intramolecular aldol condensation. With this idea in mind, we initiated a project directed at studying the cyclization of **7a** and related substrates **7b** and **7c** with a view to obtaining tricyclic intermediates suitable for the synthesis of forskolin.[8]

β-Keto esters **7a**, **7b**, and **7c** were easily obtained by alcoholysis of the acetyl derivative of Meldrum's acid (**9**)[9] with the corresponding hydroxyl derivatives **8a**, **8b**, and **8c**, respectively. In turn, **8b** and **8c** were prepared by known procedures,[10,11] whereas **8a** was prepared from α-cyclocitral by a four-step sequence.[12]

Unfortunately, under a variety of reaction conditions, **7a** and **7b** produced either recovered starting materials, products of side reactions (transesterifications or eliminations), or complex mixtures. However, the treatment of **7c** with potassium carbonate in ethanol at room temperature[13] afforded lactone **10** in 63% yield together with a small amount of the hemiacetal **11** (14% yield). While further manipulation of lactone **10** under basic conditions failed to produce the aldol product **6**, treatment of **10** with *p*-toluenesulfonic acid in anhydrous benzene (5 h, 25 °C) produced the desired tricyclic α,β-unsaturated ketone **6** in 75% yield along with 8% of the enol ether **12**. Longer exposure of **10** to the above reaction conditions (48 h) yielded the β,γ-unsaturated ketone **13** as the main product. This tricyclic unconjugated ketone **13** was previously ob-



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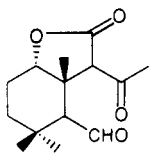

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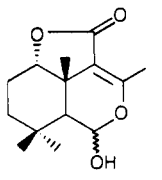

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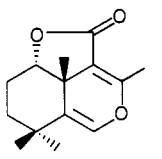

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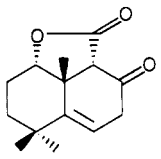

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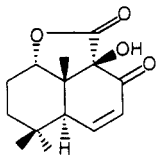

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tained by a different route in an unsuccessful attempt to prepare **6**.[5j] In agreement with previous observations,[5j]

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(8) Long after the initiation of our project appeared the publication of Koft et al.[5h] reporting a conceptually similar approach to that described in this paper.

(9) Oikawa, Y.; Sugano, K.; Yonemitsu, O. *J. Org. Chem.* **1978**, *43*, 2087.

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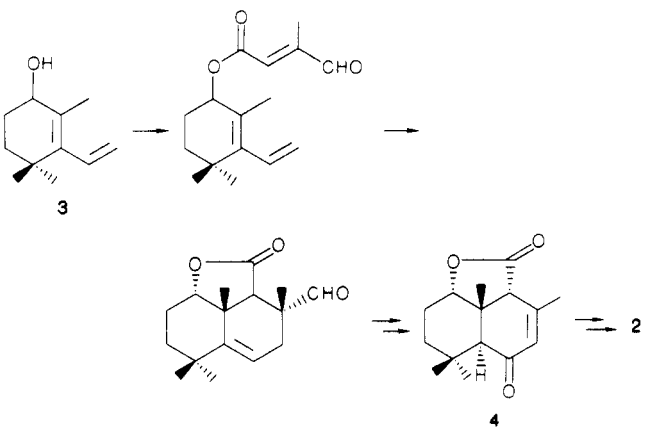
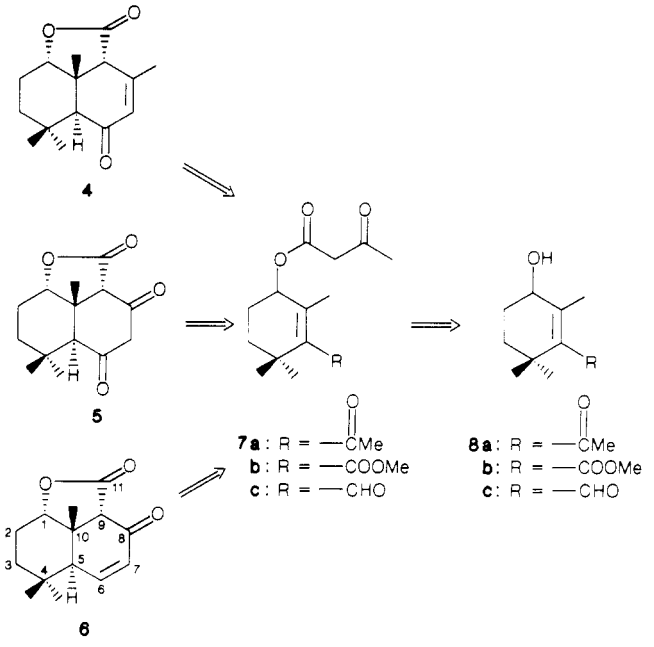
Scheme I

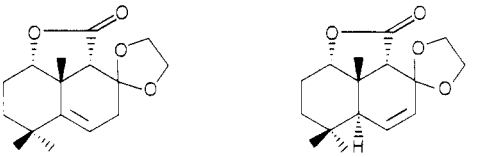


Scheme II



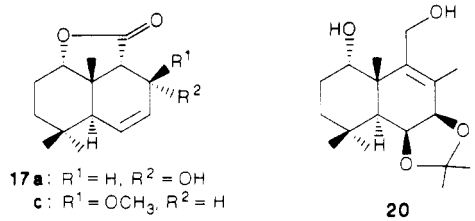
attempts to isomerize **13** into **6** under various reaction conditions failed. However, by careful control of the reaction time of the acid-catalyzed aldol condensation of **10**, **6** or **13** can selectively be obtained. The structure of **6** is supported by an exhaustive spectral analysis. The ^{1}H NMR spectrum shows the typical pattern of signals, readily attributable to the α,β-unsaturated ketone moiety, as double doublets at δ 6.13 (J = 10.1 and 2.95 Hz, H-7) and 7.10 (J = 10.1 and 2.95 Hz, H-6), and the ^{13}C NMR signal at 188.2 ppm is compatible with a conjugated ketone. Furthermore, the relative stereochemistry of **6** follows conclusively from a nuclear Overhauser enhancement (NOE) of the H-1 (δ 4.30) and H-9 (δ 3.10) signals observed upon irradiation at δ 1.31 (C-10 Me), whereas the signal of H-5 (δ 2.45) is unaffected. The failure of substrates **7a** and **7b** to cyclize in the desired manner could be attributed to the less efficient conjugation of the acetyl and carbomethoxyl groups, due to a loss of coplanarity with the adjacent double bond.

With access to the tricyclic ketone **6** secure, we next focused on the preparation of an intermediate that could be useful for the synthesis of **4** and, eventually, of the Ziegler intermediate **2**. In a series of exploratory experiments, we first attempted the direct functionalization of the double bond of **6**. We have found that the treatment of **6** with osmium tetraoxide, together with *N*-methylmorpholine *N*-oxide as a stoichiometric oxidant,[14] led to a product characterized as **14**[15] in 70% yield. The formation of **14** suggests that the enolate of **6** is selectively attacked by the oxidant.[16] In order to avoid the apparently easy enolate formation in **6**, we decided to study the blocking of its keto carbonyl group. However, in acid-catalyzed ketalization of **6**, even using a procedure by which no double bond migration has been reported in related systems,[17] a 4:1 ratio (^{1}H NMR) of the undesired ketal **15** to the known **16**[5j] was obtained. Our attempts



to prepare the corresponding thioketal were also unsuccessful. Although sodium borohydride reduction of **6** afforded mainly a saturated alcohol, we found that sodium borohydride in the presence of cerium(III) chloride[18] gave a mixture of epimeric allylic alcohols in good yield. By silica gel column chromatography of this mixture, the pure alcohols **17a** and **17b**, in a 2:1 ratio, were obtained.

The stereochemical assignment of these alcohols follows from an exhaustive analysis of their NMR spectra. In the ^{1}H NMR spectrum of **17a**, the one-proton signal at δ 2.70 assigned to the methine at C-9, which appears as a doublet with a J value of 9 Hz, indicated that the hydroxyl group is pseudoequatorial. Additionally, in the spectrum of **17b**, with the hydroxyl group pseudoaxial, the doublet at δ 2.49 with a J value of 2 Hz, assigned again to the methine at C-9, confirms the above attributions. Further support for the stereochemical assignment of **17b** and, consequently, of **17a** was obtained by conversion of **17b** into the recently reported methyl ether **17c**.[19] It is worthwhile mentioning that **17c** was synthesized by Ikegami et al.,[7b] by an intramolecular Diels–Alder strategy in one of the recently successful approaches to forskolin (1).



(12) (1) MeMgI, Et_2O, 0 °C, 1 h; (2) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −35 °C, 30 min; (3) MCPBA, CH_2Cl_2, room temperature, 1 h; (4) NaH, THF–DMF, room temperature, 3 h.

(13) Stork, G.; Taber, D. F.; Marx, M. *Tetrahedron Lett.* **1978**, 2445.

(14) Van Rheenan, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973.

(15) **14**: IR ν 3420, 2990, 2910, 2900, 2860, 1780, 1665, 1605, 1450, 1380, 1350, 1270, 1210, 1180, 1150, 1120, 1010, 970, 910, 830 cm^{-1}; ^{1}H NMR δ 7.23 (dd, J = 9.6 and 3.2 Hz, H-6), 6.22 (dd, J = 9.6 and 3.2 Hz, H-7), 4.64 (t, J = 3.2 Hz, H-1), 4.34 (s, OH), 2.49 (t, J = 3.2 Hz, H-5), 2.02 (m, H-2), 1.51 (m, H-3), 1.15 (10-Me), 1.06 (4-Me); ^{13}C NNR δ 192.0 (C-8), 171.5 (CO, lactone), 152.5 (C-6), 128.5 (C-7), 82.9 (C-9), 81.2 (C-1), 52.8 (C-10), 45.5 (C-5), 33.9 (C-3), 31.7 (C-4), 31.3 (4α-Me), 21.8 (4β-Me), 20.7 (C-2), 9.8 (10-Me).

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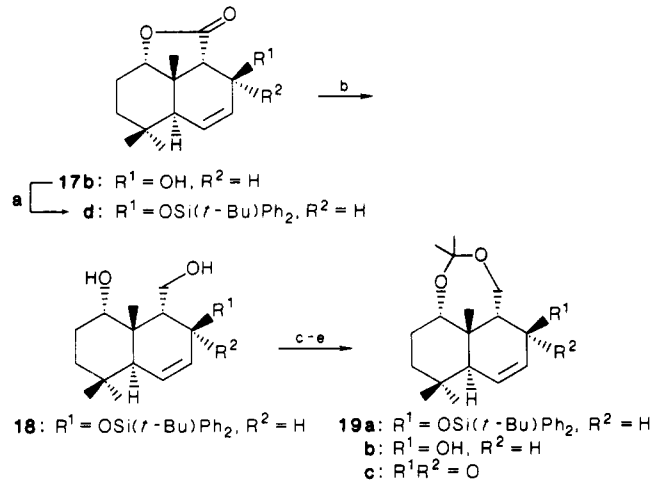
(17) Petersen, Q. R.; Sowers, E. E. *J. Org. Chem.* **1964**, *29*, 1627.

(18) Luche, J. L.; Gamal, A. L. *J. Am. Chem. Soc.* **1979**, *101*, 5848.

(19) Methyl ether **17c**: mp 80–81 °C (lit.[7b] mp 81–82 °C); IR ν 3030, 2940, 2960, 2860, 1760, 1480, 1460, 1450, 1380, 1370, 1345, 1255, 1200, 1175, 1150, 1110, 1085, 1035, 990, 980, 960, 865, 810 cm^{-1}; ^{1}H NMR δ 5.89 (br s, H-6 and H-7), 4.33 (t, J = 3.4 Hz, H-1), 3.99 (m, H-8), 3.46 (s, OMe), 2.44 (d, J = 1.4 Hz, H-9), 1.98 (m, H-2), 1.39 (m, H-3), 1.13 (Me), 0.94 (6 H, Me).

Scheme III[a]



[a] Reagents and conditions: (a) (*t*-Bu)Ph_2SiCl, imidazole, DMF, 50 °C; (b) $LiAlH_4$, THF, 20 °C; (c) $Me_2C(OMe)_2$, TsOH catalyst, 20 °C; (d) *n*-Bu_4NF, THF, 20 °C; (e) PCC, CH_2Cl_2, 20 °C.

In spite of the fact that having established a simple synthetic route to a key intermediate such as **17c** would fulfill our initial objective, it was attractive to investigate further the use of alcohols **17a** and/or **17b** in the development of shorter routes to more advanced intermediates than **17c** and used previously in the total synthesis of forskolin (**1**).[7] As an example, we easily converted **17b** into acetonide **19c** by a simple and high-yield sequence (Scheme III). Interestingly enough, **19c**, prepared by a cation-mediated polyene cyclization, has been transformed into **20**,[5j] which in turn has also been prepared from the Ziegler intermediate (**2**).[7b] Although we arbitrarily have chosen **17b** as starting material, the same result should be obtained with **17a** or with the mixture of both allylic alcohols.

In conclusion, the sequence described in this report, apart from providing simple and efficient entries to precursors for the synthesis of a complex natural product such as forskolin (**1**), shows that the intramolecular Michael addition–aldol condensation sequence is a valuable alternative to the Diels–Alder addition for the construction of highly functionalized molecules.

Experimental Section

Melting points were determined on a hot-stage microscope and are uncorrected. IR spectra were measured as solids in KBr disks. NMR spectra were recorded at 80 MHz in $CDCl_3$ solutions. The ^{13}C NMR spectra were measured at 20.15 MHz. Column chromatography was performed on silica gel 60H, slurry packed, run under low pressure of nitrogen and employing increasing amounts of EtOAc in hexane as solvent, unless otherwise stated. Analytical thin-layer chromatography (TLC) was conducted on Merck aluminum plates precoated with 0.2 mm of silica gel 60 F-254. The numbering sequence used for reporting NMR parameters is illustrated in structure **6**.

(2aβ,5aα,8aβ,8bβ)-2a,3,5a,6,7,8,8a,8b-Octahydro-3-oxo-6,6,8b-trimethyl-2*H*-naphtho[1,8-*bc*]furan-2-one (6). A solution of hydroxy aldehyde **8c** (800 mg, 4.75 mmol) and acetyl Meldrum's acid (972 mg, 5.23 mmol) in anhydrous benzene (25 mL) was heated at reflux for 3 h. The cooled solution was washed with 10% aqueous $NaHCO_3$ (2 × 15 mL), H_2O, and brine, dried (Na_2SO_4), and evaporated. Chromatography of the crude product resulted in the isolation of the oily β-keto ester **7c** (1.1 g, 92%). TLC analysis verified the purity of the product: IR ν 2960, 2860, 1745, 1720, 1680, 1415, 1360, 1310, 1270, 1240, 1150, 1035, 1010, 995, 970, 835 cm^{-1}; ^1H NMR δ 10.15 (s, CHO), 5.36 (t, J = 5.2 Hz, HCOR), 3.53 (s, $COCH_2CO$), 2.28 (s, MeCO), 2.05 (d, J = 0.9 Hz, =CMe), 1.24, 1.19 (Me); ^{13}C NMR δ 199.8 (MeC=O), 192.8 (CHO) 166.5 (COO), 147.2 (=*C*Me), 143.5 (=CCHO), 73.3 (HCOR), 49.9 ($COCH_2CO$), 35.5 (CH_2CMe_2), 33.3 (CMe_2), 30.0 (CH_2COR), 27.3, 26.7 (CMe_2), 24.5 (CH_3CO), 15.3 (=CCH_3). This material was used immediately for the next step. Following the same procedure as in preparing **7c**, **8a**, and **8b** gave **7a** and **7b**, respectively. β-Keto ester **7a**: ^1H NMR δ 5.23 (t, J = 4.5 Hz, HCO), 3.48 (s, $COCH_2CO$), 2.30 (s, =CCOMe), 2.27 (s, CH_2COMe), 1.59 (br s, =CMe), 1.10 (s, 6 H, Me). β-Keto ester **7b**: ^1H NMR δ 5.26 (t, J = 4.5 Hz, HCO), 3.76 (s, COOMe), 3.47 (s, $COCH_2CO$), 2.26 (s, COMe), 1.64 (s, =CMe), 1.10 (s, 6 H, Me).

To a well-stirred mixture of anhydrous K_2CO_3 (5 g, 36 mmol) in anhydrous EtOH (150 mL) at room temperature was added dropwise a solution of **7c** (1.1 g, 4.36 mmol) in anhydrous EtOH (50 mL). The mixture was stirred for 4 h, after which the solvent was evaporated. The residue was dissolved in cold H_2O, carefully acidified with 10% aqueous HCl (with cooling) to pH 5, and extracted. The $CHCl_3$ extracts were washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed to yield **10** (692 mg, 63%) and **11** (154 mg, 14%). Compound **10** proved to be prone to decomposition and so was used immediately for the next step: IR ν 3405, 2930, 2875, 1775, 1711, 1685, 1650, 1460, 1360, 1301, 1211, 1180, 1040, 992 cm^{-1}; ^1H NMR δ 10.03 (d, J = 1.7 Hz, CHO), 4.60 (t, 3 Hz, HCO), 3.95 (s, COCHCO), 2.34 (s, COMe), 1.91 (d, J = 1.7 Hz, *CH*CHO), 1.26, 1.22, 1.19 (s Me); ^{13}C NMR δ 204.1 (CHO), 202.8 (*C*OMe), 172.4 (CO lactone), 82.7 (C-1), 65.9 (C-9), 59.4 (C-5), 44.6 (C-10), 32.9 (C-3), 32.7 (4α-Me), 31.9 (C-4), 31.7 (C-7), 21.8 (4β-Me), 20.2 (C-2), 14.7 (10-Me); mass spectrum, m/e (relative intensity) 252 (M^+, 8), 237 (5), 224 (18), 209 (13), 182 (11), 168 (18), 153 (43), 139 (78), 123 (100), 111 (59), 98 (39), 85 (60), 81 (38), 69 (31), 55 (19), 43 (97). Compound **11**: mp 176.5–177.5 °C (EtOAc); IR ν 3360, 2960, 2920, 2860, 1725, 1660, 1400, 1380, 1315, 1300, 1240, 1220, 1175, 1110, 1035, 1015, 990, 965, 830 cm^{-1}; ^1H NMR δ 5.54 (br s, H-6), 4.16 (m, H-1), 3.42 (br s, OH), 2.20 (s, 8-Me), 1.86 (s, H-5), 1.43 (10-Me), 1.04, 0.78 (4-Me); ^{13}C NMR [$(CD_3)_2CO$] δ 174.2 (CO, lactone), 159.8 (C-8), 103.7 (C-9), 95.0 (C-6), 84.8 (C-1), 49.7 (C-5), 36.9 (C-10), 36.5 (C-3), 32.6 (C-4), 32.4 (4α-Me), 29.8 (10-Me), 26.9 (C-2), 21.3 (4β-Me), 16.2 (8-Me); mass spectrum, m/e (relative intensity) 252 (M^+, 27), 237 (23), 224 (39), 209 (11), 191 (10), 167 (10), 163 (23), 153 (100), 140 (92), 123 (100), 122 (42), 111 (26), 107 (25), 91 (20), 85 (96), 69 (44), 55 (59); found for M^+ m/e 252.1371 ($C_{14}H_{20}O_4$ requires m/e 252.1362).

To a well-stirred solution of *p*-TsOH in anhydrous benzene (130 mL) at room temperature [prepared from *p*-TsOH·H_2O (1.04 g, 5.47 mmol) dried by azeotropic distillation with 250 mL of benzene] was added a solution of **10** (690 mg, 2.74 mmol) in anhydrous benzene (20 mL) dropwise in 15 min. The mixture was stirred vigorously for 5 h until the TLC spot for the starting material had disappeared. The solution was washed with H_2O until neutral, then with brine, dried (Na_2SO_4), and evaporated. The solid residue was recrystallized from benzene–Et_2O to yield **6** (481 mg, 75%) as white needles: mp 152.5–154 °C; IR ν 3030, 2930, 2850, 1763, 1673, 1380, 1460, 1250, 1150, 1097, 1026, 951 cm^{-1}; ^1H NMR δ 7.10 (dd, J = 2.95 and 10.1 Hz, H-6), 6.13 (dd, J = 2.95 and 10.1 Hz, H-7), 4.30 (t, J = 3.4 Hz, H-1), 3.10 (s, H-9), 2.45 (t, J = 2.95 Hz, H-5), 2.02 (m, H-2), 1.50 (m, H-3), 1.31 (10-Me), 1.04, 1.05 (4-Me); ^{13}C NMR δ 188.2 (C-8), 169.3 (CO, lactone), 149.7 (C-6), 129.6 (C-7), 81.7 (C-1), 63.9 (C-9), 47.9 (C-10), 44.8 (C-5), 34.1 (C-3), 30.9 (4α-Me), 30.8 (C-4), 21.2 (4β-Me), 21.1 (C-2), 17.9 (10-Me); mass spectrum, m/e (relative intensity) 234 (M^+, 15), 219 (13), 206 (12), 175 (7), 147 (7), 133 (10), 120 (53), 109 (100), 91 (26), 81 (38), 79 (26), 77 (27); found for M^+ m/e 234.1256 ($C_{14}H_{18}O_3$ requires m/e 234.1256). Chromatography of the mother liquors afforded **12** (52 mg, 8%) as colorless prisms (hexane): mp 81–81.5 °C; IR ν 2920, 2860, 1745, 1680, 1620, 1450, 1365, 1350, 1290, 1220, 1205, 1150, 1110, 1050, 1025, 1000, 935, 925, 890, 870, 830, 780, 760, 740 cm^{-1}; ^1H NMR δ 6.51 (s, H-6), 4.20 (m, H-1), 2.30 (s, 8-Me), 1.28, 1.17, 1.15 (Me); ^{13}C NMR δ 169.0 (CO, lactone), 161.2 (C-8), 138.8 (C-6), 128.2 (C-5), 105.2 (C-9), 84.3 (C-1), 38.1 (C-10), 35.8 (C-3), 34.1 (4α-Me), 32.4 (C-4), 30.5 (4β-Me), 28.8 (10-Me), 26.3 (C-2), 15.5 (8-Me).

(2aβ,8aβ,8bβ)-2a,3,4,6,7,8,8a,8b-Octahydro-3-oxo-6,6,8b-trimethyl-2*H*-naphtho[1,8-*bc*]furan-2-one (13). When the same procedure described above for converting **10** to **6** was fol-

lowed, but with 48 h of stirring at room temperture, **10** (1.1 g, 4.36 mmol) afforded **13** (830 mg, 81%) as a colorless foam: ^{1}H NMR δ 5.81 (dd, J = 4.5 and 3.2 Hz, H-6), 4.35 (t, J = 3 Hz, H-1), 3.21 (dd, J = 23 and 4.5 Hz, H-7), 3.21 (s, H-9), 2.85 (dd, J = 23 and 3.2 Hz, H-7), 2.06 (m, H-2), 1.41 (10-Me), 1.19 (s, 6 H, 4-Me); NOE irradiation at δ 1.41 enhanced δ 4.35 and 3.21; ^{13}C NMR δ 201.6 (C-8), 170.3 (CO, lactone), 144.0 (C-5), 119.4 (C-6), 84.4 (C-1), 64.6 (C-9), 47.7 (C-10), 38.2 (C-7), 35.2 (C-4), 33.0 (C-3), 30.7 (4α-Me), 27.7 (10-Me), 22.6 (4β-Me), 20.9 (C-2); mass spectrum, m/e (relative intensity) 234 (M^+, 47), 219 (25), 201 (24), 189 (57), 175 (100), 159 (27), 149 (29), 147 (29), 145 (29), 133 (72), 121 (25), 105 (49), 93 (22), 91 (68), 79 (46), 77 (55); found for M^+ m/e 234.1273 ($C_{14}H_{18}O_3$ requires m/e 234.1256).

Allylic Alcohols 17a and 17b. To a well-stirred solution of **6** (320 mg, 1.37 mmol) and $CeCl_3 \cdot 7H_2O$ (510 mg, 1.37 mmol) in MeOH (10 mL) at room temperature was added $NaBH_4$ (57 mg, 1.5 mmol) in one portion. A vigorous evolution of gas together with a spontaneous rise in temperature was observed. The resulting solution was stirred at room temperature for 15 min, after which it was neutralized with dilute HCl and extracted with Cl_3CH. The combined organic extracts were washed with brine, dried (Na_2SO_4), and evaporated. Chromatography of the residue gave, in order of elution, alcohols **17a** (192 mg, 59.5%) and **17b** (89 mg, 27.5%).

3α-Hydroxy-(2aβ,5aα,8aβ,8bβ)-2a,3,5a,6,7,8,8a,8b-octahydro-6,6,8b-trimethyl-2*H*-naphtho[1,8-*bc*]furan-2-one (17a): mp 149.5–150 °C (EtOAc–Et_2O); IR ν 3460, 3020, 2920, 2860, 1750, 1675, 1460, 1380, 1360, 1210, 1180, 1155, 1050, 1035, 990, 970, 950, 830, 810 cm^{-1}; ^{1}H NMR δ 5.93 (m, H-6 and H-7), 4.63 (m, $W_{1/2}$ = 15 Hz, H-8), 4.25 (t, J = 4 Hz, H-1), 2.70 (d, J = 9 Hz, H-9), 2.18 (br s, H-5), 1.98 (m, H-2), 1.45 (m, H-3), 1.19 (10-Me), 0.98, 0.94 (4-Me); ^{13}C NMR δ 177.6 (CO, lactone), 129.4, 129.0 (C-6 and C-7), 82.4 (C-1), 63.9 (C-8), 53.6 (C-9), 47.0 (C-10), 42.3 (C-5), 34.3 (C-3), 31.0 (4α-Me), 30.9 (C-4), 21.8 (C-2), 21.4 (4β-Me), 18.4 (10-Me); mass spectrum, m/e (relative intensity) 236 (M^+, 25), 218 (12), 193 (12), 177 (21), 159 (100), 147 (21), 136 (23), 135 (38), 133 (26), 131 (25), 124 (56), 123 (60), 122 (46), 121 (78), 119 (33), 118 (49), 117 (61), 111 (44), 107 (71), 105 (40), 95 (42), 93 (44), 91 (64), 81 (49), 77 (50); found for M^+ m/e 236.1403 ($C_{14}H_{20}O_3$ requires 236.1412).

3β-Hydroxy-(2aβ,5aα,8aβ,8bβ)-2a,3,5a,6,7,8,8a,8b-octahydro-6,6,8b-trimethyl-2*H*-naphtho[1,8-*bc*]furan-2-one (17b): mp 106.5–107.5 °C (EtOAc); IR ν 3420, 3010, 2920, 2880, 1765, 1465, 1380, 1340, 1270, 1180, 1150, 1050, 1030, 995, 970, 955, 800 cm^{-1}; ^{1}H NMR δ 5.87 (m, H-6 and H-7), 4.49 (m, $W_{1/2}$ = 6 Hz, H-8), 4.34 (t, J = 3.2 Hz, H-1), 3.22 (br s, OH), 2.49 (d, J = 2 Hz, H-9), 1.97 (m, H-2), 1.58 (br s, H-5), 1.39 (m, H-3), 1.17 (10-Me), 0.94 (s, 6 H, 4-Me); ^{13}C NMR δ 178.2 (CO, lactone), 130.9, 127.2 (C-6 and C-7), 82.5 (C-1), 64.8 (C-8), 61.7 (C-9), 43.7 (C-5), 40.6 (C-10), 34.3 (C-3), 31.2 (4α-Me), 30.8 (C-4), 21.2 (C-2), 20.7 (4β-Me), 18.9 (10-Me); mass spectrum found for M^+ m/e 236.1412 ($C_{14}H_{20}O_3$ requires 236.1412).

(4aβ,7aα,10aβ,10bβ)-4a,5,7a,8,9,10,10a,10b-Octahydro-5-oxo-2,2,8,8,10b-pentamethylnaphtho[1,8-*de*]-1,3-dioxepane (19c). To a stirred solution of alcohol **17b** (70 mg, 0.3 mmol) and imidazole (88 mg, 1.3 mmol) in anhydrous DMF was added *tert*-butyldiphenylsilyl chloride (0.17 mL, 0.66 mmol). After 3 h of stirring at 50 °C, the solution was cooled, poured into H_2O (10 mL), and extracted with $CHCl_3$. The combined organic extracts were washed with dilute HCl and brine, dried (Na_2SO_4), and evaporated. The crude residue was used in the next step without purification; a small sample was chromatographed to afford **17d** as a colorless syrup: IR ν 3060, 3030, 2950, 2915, 2860, 1770, 1590, 1480, 1470, 1435, 1370, 1260, 1180, 1150, 1070, 1040, 1000, 990, 960, 870, 820, 750, 700, 640, 610 cm^{-1}; ^{1}H NMR δ 7.70 (m, 4 H), 7.38 (m, 6 H), 5.63 (m, H-6 and H-7), 4.63 (m, H-8), 4.25 (t, J = 3.2 Hz, H-1), 2.60 (br s, H-9), 1.54 (m, H-5), 1.22 (10-Me), 1.09 (Me), 1.07 (s, 12 H, *t*-Bu and Me); ^{13}C NMR δ 176.5 (CO, lactone), 135.6, 134.6, 130.9, 129.7, 129.5, 128.1, 127.5, 125.9 (C-6, C-7, and Ph), 81.9 (C-1), 65.8 (C-8), 61.6 (C-9), 43.0 (C-5), 39.9 (C-10), 34.3 (C-3), 31.0 (4α-Me), 30.9 (C-4), 26.8 (CMe_3), 21.2 (C-2), 20.7 (4β-Me), 19.1 (CMe_3), 18.4 (10-Me).

To a stirred mixture of $LiAlH_4$ (12 mg, 0.31 mmol) in anhydrous THF (1 mL) was added a solution of crude **17d** also in anhydrous THF (1 mL) at room temperature. After 2 h, the reaction mixture was quenched with acid and extracted with Et_2O. The ethereal solution was washed with brine, dried (Na_2SO_4), and evaporated. The residue was used in the next step without purification. A small sample was chromatographed, affording **18** as a foam: IR ν 3220, 3030, 3010, 2880, 2840, 1660, 1600, 1470, 1460, 1430, 1390, 1370, 1270, 1220, 1200, 1170, 1110, 1060, 1020, 980, 860, 830, 750, 705, 615 cm^{-1}; ^{1}H NMR δ 7.70 (m, 4 H), 7.40 (m, 6 H); 5.60 (m, H-6 and H-7), 3.84 (m, $W_{1/2}$ = 4 Hz, H-1), 3.65 (m, $W_{1/2}$ = 4 Hz H-8), 3.52 (dd, J = 11.8 and 8.7 Hz, H-11 B), 2.92 (dd, J = 11.8 and 2.0 Hz, H-11 A), 2.20 (m, H-5), 1.58 (m, H-9), 1.23 (10-Me), 1.07 (*t*-Bu), 0.98, 0.93 (4-Me); ^{13}C NMR δ 135.9, 135.0, 134.8, 134.3, 133.6, 129.5, 129.4, 128.7, 128.3, 127.7, 127.5 (C-6, C-7, and Ph), 73.4, 72.1 (C-1 and C-8), 63.3 (C-11), 57.5 (C-9), 40.4 (C-5), 39.7 (C-10), 34.2 (C-3), 32.8 (4α-Me), 32.7 (C-4), 26.9 (CMe_3), 26.5 (C-2), 23.1 (10-Me), 22.9 (4β-Me), 19.1 (CMe_3).

To a stirred solution of crude **18** in 2,2-dimethoxypropane (0.2 mL) was added a crystal of *p*-TsOH at room temperature. After 3 h, the reaction mixture was diluted with Et_2O (10 mL) and washed with saturated $NaHCO_3$ solution and brine. After drying (Na_2SO_4) and removal of the solvent, the residue was chromatographed to give **19a** (64 mg, 83% from **17b**) as a solid: mp 134–137 °C (hexane); IR ν 3060, 3030, 2980, 2960, 2940, 2860, 1660, 1590, 1465, 1440, 1380, 1220, 1175, 1115, 1080, 1060, 1035, 1010, 980, 940, 860, 820, 745, 700, 680, 620 cm^{-1}; ^{1}H NMR δ 7.68 (m, 4 H), 7.39 (m, 6 H), 5.68 (br d, J = 10.2 Hz, H-6), 5.37 (br dt, J = 10.2 and 3 Hz, H-7) 3.74 (br d, J = 2.4 Hz, H-1), 3.63 (m, H-8), 3.43 (q, J = 12.0 Hz, H-11 B), 2.90 (dd, J = 12.0 and 3.15 Hz, H-11 A), 2.28 (m, H-5), 1.79 (m, H-9), 1.32 (Me), 1.21 (2 Me), 1.06 (*t*-Bu), 0.94 (2 Me); ^{13}C NMR δ 135.9, 134.75, 134.65, 133.9, 129.5, 129.4, 128.3, 127.8, 127.6, 127.4 (C-6, C-7, and Ph) 99.6 (OCO), 72.8 (C-1) 68.6 (C-8), 62.2 (C-11), 58.0 (C-9), 39.9 (C-5), 37.3 (C-10), 34.8 (C-3), 33.2 (4α-Me), 32.2 (C-4), 27.0 (CMe_3), 25.6 (Me, acetonide), 25.1 (C-2), 24.2 (Me, acetonide), 23.3 (10-Me), 22.1 (4β-Me), 19.1 (CMe_3).

A solution of **19a** (60 mg, 0.115 mmol) in a 1 M solution of tetra-*n*-butylammonium fluoride (0.5 mL) was stirred at room temperature for 36 h. After removal of the solvent, the residue was chromatographed to give **19b** (32.5 mg, 100%) as colorless needles (hexane): mp 117.5–118.5 °C; IR ν 3400, 3010, 2970, 2930, 2850, 1660, 1465, 1380, 1230, 1175, 1150, 1070, 1050, 1010, 980, 900, 870, 780, 705 cm^{-1}; ^{1}H NMR δ 5.75 (m, H-6 and H-7), 3.58–3.88 (m, H-1, H-8, and H-11 B), 3.29 (dd, J = 12.0 and 3.2 Hz, H-11 A), 2.35 (m, H-5), 1.34 (Me), 1.29 (Me), 1.07 (Me), 1.00 (Me), 0.92 (Me); ^{13}C NMR δ 129.5, 127.4 (C-6 and C-7), 99.7 (OCO), 72.5 (C-1), 67.5 (C-8), 62.7 (C-11), 58.3 (C-9), 40.1 (C-5), 37.1 (C-10), 34.7 (C-3), 33.1 (4α-Me), 32.2 (C-4), 25.5 (Me, acetonide), 25.0 (C-2), 24.0 (Me, acetonide), 23.2 (10-Me), 22.3 (4β-Me).

To a well-stirred suspension of PCC[20] (97 mg, 0.45 mmol) and anhydrous NaOAc (5.3 mg, 0.064 mmol) in anhydrous CH_2Cl_2 (2 mL) was added acetonide **19b** (16 mg, 0.057 mmol). After 45 min of stirring at room temperature, the mixture was decanted and the residue washed several times with CH_2Cl_2. The combined organic extracts were filtered through a short silica gel pad and evaporated. The residue was chromatographed on a silica column (hexane and mixtures of hexane–CH_2Cl_2) to afford pure **19c** (14 mg, 87%) as a colorless oil, which crystallized on standing: mp 76–78 °C (lit.[5j] mp 80–81.5 °C); IR ν 3030, 2950, 2930, 2860, 1740, 1660, 1460, 1450, 1440, 1370, 1260, 1230, 1170, 1140, 1100, 1075, 1050, 1030, 1010, 965, 890, 880, 840, 810, 780 cm^{-1}; ^{1}H NMR δ 6.96 (dd, J = 10.3 and 2.2 Hz, H-6), 5.96 (dd, J = 10.3 and 3.3 Hz, H-7), 3.95 (dd, J = 12.1 and 10.7 Hz, H-11 B), 3.72 (m, H-1), 3.37 (dd, J = 12.1 and 3.4 Hz, H-11 A), 2.98 (dd, J = 3.3 and 2.2 Hz, H-5), 2.27 (dd, J = 10.7 and 3.4 Hz, H-9), 1.34 (s, 6 H), 1.10 (Me), 1.05 (Me), 0.98 (Me); ^{13}C NMR δ 198.6 (C-8), 151.2 (C-6), 128.6 (C-7), 100.6 (OCO), 72.5 (C-1), 64.8 (C-9), 59.7 (C-11), 43.8 (C-5), 42.4 (C-10), 34.5 (C-3), 33.0 (4α-Me), 32.1 (C-4), 25.6 (Me, acetonide), 24.7 (C-2), 24.0 (Me, acetonide), 23.6 (10-Me), 21.9 (4β-Me). These spectral data are coincident with those reported in ref 5j.

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Note added in proof: After the submission of this manuscript for publication, a similar approach to compound **6** appeared in the literature (Li, T.-T.; Wu, Y.-L. *Tetrahedron Lett.* **1988**, *29*, 4039).

Registry No. (±)-**1**, 112420-42-5; (±)-**6**, 118798-10-0; (±)-**7a**, 118798-07-5; (±)-**7b**, 118798-08-6; (±)-**7c**, 118798-09-7; (±)-**8d**, 118798-06-4; (±)-**8b**, 60078-94-6; (±)-**8c**, 60078-92-4; **9**, 72324-39-1; **10**, 118798-11-1; **11**, 118798-12-2; (±)-**12**, 118798-13-3; (±)-**19**, 118798-14-4; (±)-**14**, 118798-15-5; (±)-**15**, 118798-16-6; (±)-**16**, 118798-17-7; (±)-**17a**, 118798-18-8; (±)-**17b**, 118916-42-0; (±)-**17c**, 114375-37-0; (±)-**17d**, 118798-19-9; (±)-**18**, 118798-21-3; (±)-**19a**, 118798-22-4; (±)-**19b**, 118798-23-5; (±)-**19c**, 118798-20-2; (±)-α-cyclocitral, 59462-59-8; α,2,6,6-tetramethyl-2-cyclohexene-1-methanol, 118798-24-6; (±)-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)ethanone, 72717-26-1; 1-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]-hept-2-yl)ethanone, 118798-25-7.

Purification and Inhibition of Spinach α,β-Dihydroxyacid Dehydratase†

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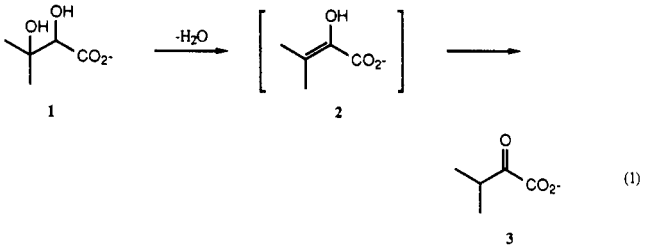
The α,β-dihydroxyacid dehydratase (E.C. 4.2.1.9) responsible for the production of α-oxoisovaleric acid in the valine biosynthetic pathway has been purified from spinach leaves. Its properties are similar to those given in a previous report using a less pure preparation. Its monomer mass is estimated to be 55 kDa. Evidence for an enol intermediate in the reaction mechanism has been obtained by a deuterium labeling study. Several inhibitors have been screened against the enzyme. Four of particular effectiveness are 4-fluoro-2,3-dihydroxyisovaleric acid, 1-hydroxy-1-isobutanesulfonic acid, *N,N*-dimethylglycine *N*-oxide, and 2-fluoro-3,3-dimethylacrylic acid. As an enol analogue, the latter compound gives further evidence for an enol intermediate.

The biosynthetic pathway for the branched-chain amino acids valine, leucine, and isoleucine in higher plants has recently been identified as a site of herbicide action. Three classes of compounds, the sulfonylureas,[1-4] imidazolinones,[5,6] and triazolopyrimidines,[7] have been show to inhibit the first and rate-limiting enzyme in the pathway, E.C. 4.1.3.18, acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS). These compounds have found commercial success as soybean and small grain herbicides, but still lack selectivity for grasses, and some resist soil metabolism. Consequently, two other enzymes in this pathway draw attention as potential targets for developing new meristematic inhibitors. The acetohydroxyacid reductioisomerase[8] has thus far not been subjected to serious scrutiny. This work has focused on the subsequent enzyme in the pathway, α,β-dihydroxyacid dehydratase (DHAD). This enzyme catalyzes the transformation of 2,3-dihydroxyisovaleric acid (**1**) into 2-oxoisovaleric acid (**3**) with loss of water (eq 1).



1 2 3 (1)

Much of the detailed information about the valine biosynthetic pathway has come from studies on bacterial enzymes. DHAD has been partially purified from *Escherichia coli*,[9] *Neurospora crassa*,[10] and *Salmonella typhimurium*.[11] The stereochemical course of the *E. coli*[12] and *Salmonella*[13] enzymes has been well-studied. It has been shown that a 2*R* configuration is uniformly required for both the natural substrates and analogues. Evidence from tritium labeling studies implicates an enol intermediate in the reaction catalyzed by the *Salmonella* dehydratase.[14] DHAD has also been identified as the site of hyperbaric oxygen poisoning in *E. coli*.[15,16] It has been postulated that this is due to excessive superoxide levels, and superoxide generated from Paraquat has been shown to decrease DHAD activity in vivo.[17]

For the purpose of herbicide design, the plant enzyme is required. DHAD activity has been studied in 29 plant species[18] and has been found to strongly correlate with seedling growth. A previous publication reported the purification (120-fold, 1% activity yield) of the spinach enzyme.[8] It was shown to require Mg^{2+} for activity, as further evidenced by inhibitors such as fluoride and

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