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Total Synthesis of (+)-Conagenin

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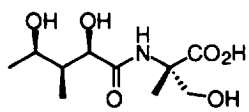
Abstract: The first total synthesis of (+)-conagenin, a novel immunomodulator produced by *Streptomyces roseosporus*, has been achieved in highly stereo- and enantioselective manner. The carboxylic acid moiety was synthesized starting with asymmetric aldol reaction of propiophenone with acetaldehyde followed by *in situ syn*-selective $NaBH_4$ reduction. The amino acid moiety was synthesized based upon Et_2AlCl catalyzed cyclization of the epoxy trichloroacetimidate prepared from (*S*)-methylglycidol. Condensation of both moieties and alkaline hydrolysis led to (+)-conagenin.

Immunomodulation is now an important therapeutic strategy for the treatment of immunodeficient diseases such as cancer, transplantation, and other immunological disorders. In 1991 Ishizuka and co-workers[1] found conagenin (**1**), a novel low molecular weight immunomodulator, in fermentation broths of *Streptomyces roseosporus* after screening for substances that exhibit specific action on T cells without activation of macrophages. Conagenin (**1**) was found to enhance the production of lymphokines responsible for thrombopoiesis and the generation of antitumor effector cells by stimulation of activated T cells.[2] It was also reported[3] that conagenin (**1**) improved the antitumor efficacy of adriamycin and mitomycin C against murine leukemias and reduced their toxicity. These findings suggest its potential utility for cancer chemotherapy. This substance posseses the densely functionalized structure consisting of a (2*R*,3*S*,4*R*)-2,4-dihydroxy-3-methylpentanoic acid moiety having three contiguous chiral centers and a (*S*)-methylserine moiety having a quaternary chiral center. The combination of the biological activity and the unique structure of conagenin (**1**) encouraged us to develop its convergent synthesis which could permit easy access to unnatural variants as well. Herein, we report the first total synthesis of conagenin (**1**) in its naturally occurring form.[4]



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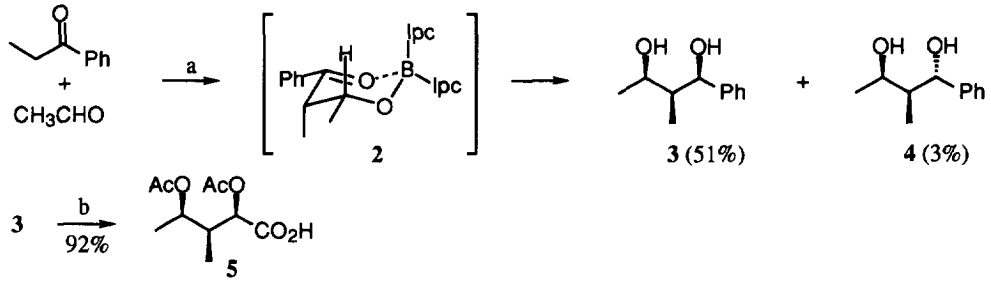
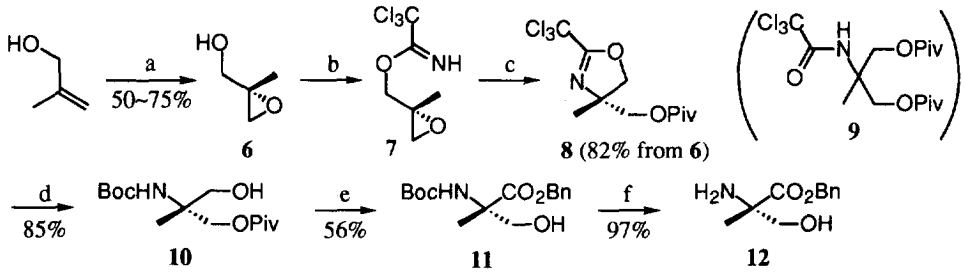
Construction of the carboxylic acid moiety relied on the sequence involving asymmetric aldol reaction[5] followed by *syn*-selective reduction.[6] Thus, propiophenone was allowed to react with (–)-diisopinocampheylboron triflate followed by acetaldehyde in dichloromethane according to the Paterson's procedure.[7] After addition of methanol[8] to the reaction mixture, the resulting chelated aldol **2** was then directly reduced[9] with $NaBH_4$ in the same flask to give diol **3**, $[\alpha]^{24}_D$ +35.5° (*c* 0.46, $CHCl_3$), and its epimer **4**, $[\alpha]^{24}_D$ –44.6° (*c* 0.40, $CHCl_3$), in a ratio of 17 to 1.[10] No other isomers having 2,3-*anti* configuration were

isolated. The absolute structures and optical purities of **3** (93% ee) and **4** (100% ee)[11] were determined respectively by ^1H NMR analysis[12] of the MTPA esters of the corresponding 1-acetoxy-3-hydroxy derivatives.[13] Upon sequential acetylation and RuO_4 mediated oxidative cleavage[14] of the phenyl group, **3** gave carboxylic acid **5**, $[\alpha]^{24}_D$ +1.6° (*c* 0.37, $CHCl_3$), in good overall yield.



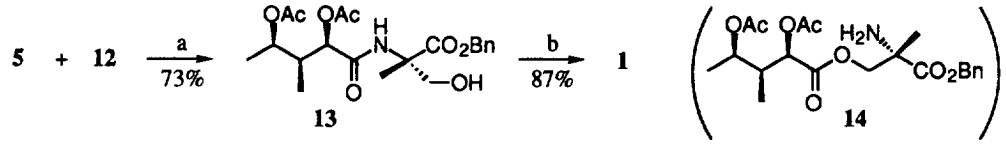
Scheme 1. (a) (–)-$(Ipc)_2BOTf$, *i*-Pr_2EtN, CH_2Cl_2, –78 ~ –23 °C, then $NaBH_4$, MeOH, –23 °C; (b) (i) Ac_2O, Et_3N-DMAP (cat.), CH_2Cl_2, (ii) $RuCl_3$ (cat.), HIO_4, CH_3CN-CCl_4-H_2O (5:5:8).

Construction of the methylserine moiety[15] was achieved based on the following methodology which centers around Lewis acid catalyzed cyclization of epoxy trichloroacetimidates.[16] Reaction of (*S*)-2-methylglycidol (**6**),[17] prepared in 94% ee[18] by the Katsuki-Sharpless catalytic asymmetric epoxidation[19] of methallyl alcohol, with trichioroacetonitrile in the presence of a catalytic amount of DBU gave trichloroacetimidate **7**. Upon treatment of **7** with 0.5 equivalent of Et_2AlCl in dichloromethane at room temperature, the cyclization did take place with complete regio- and stereoselectivity and oxazoline **8**, mp 40-44 °C; $[\alpha]^{24}_D$ –30.7° (*c* 1.00, $CHCl_3$), was obtained in 82% yield from **6** after pivaloylation. It is interesting to note that $BF_3{\cdot}Et_2O$ mediated cyclization of **7** followed by pivaloylation led to formation of a 1:2 mixture of **8** and achiral dipivalate **9** in 73% yield from **6**. Acid hydrolysis followed by *t*-butoxycarbonylation in the same flask cleanly effected transformation of **8** into alcohol **10**. Optical purity[18] of **10** (94% ee) showed that no racemization occurred during the sequence from **6** to **10**. After recrystalization from *n*-hexane, **10** became optically pure, mp 106-107 °C; $[\alpha]^{25}_D$ –8.6° (*c* 0.90, $CHCl_3$). Upon successive Swern oxidation, $NaClO_2$ oxidation, saponification, and benzylation, **10** afforded ester **11**, mp 71-72 °C; $[\alpha]^{22}_D$ +7.3° (*c* 1.00, $CHCl_3$). Exposure of **11** to trifluoroacetic acid caused quantitative de-*t*-butoxycarbonylation to give benzyl (*S*)-methylserinate (**12**), $[\alpha]^{23}_D$ –9.8° (*c* 0.66, $CHCl_3$).



Scheme 2. L-DIPT (0.06 equiv.), $Ti(O\text{-}i\text{-}Pr)_4$ (0.05 equiv.), *t*-BuOOH (2 equiv.), CH_2Cl_2, –20 °C; (b) CCl_3CN, DBU (0.1 equiv.), CH_2Cl_2, –20 °C; (c) (i) Et_2AlCl (0.5 equiv.), CH_2Cl_2, (ii) *t*-BuCOCl, Et_3N-DMAP (cat.), CH_2Cl_2; (d) 1 M HCl, THF, then $NaHCO_3$, Boc_2O; (e) (i) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, –60 to 25 °C, (ii) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH-H_2O (4:1), (iii) 2 M NaOH-EtOH (1:5), (iv) $PhCH_2Br$, K_2CO_3, DMF; (f) CF_3CO_2H, CH_2Cl_2.

Condensation of the carboxylic acid **5** with the methylserine derivative **12** was effected cleanly using a water soluble carbodiimide as a dehydrating agent in the presence of 1-hydroxybenzotriazole[20] in DMF to afford enantiomerically pure amide **13**, $[\alpha]^{23}_D$ +31.4° (*c* 0.90, $CHCl_3$).[18] Interestingly, when this reaction was carried out in dichloromethane, the corresponding *O*-acylated amine **14** (30%) was produced together with **13** (40%). However, treatment of **14** with 1 M $NaHCO_3$ in THF at room temperature resulted in its quantitative isomerization to **13**. Finally, hydrolytic removal of the ester protecting groups of **13** furnished (+)-conagenin (**1**). Synthetic substance, $[\alpha]^{25}_D$ +48.7° (*c* 0.43, MeOH), was identical with natural conagenin, $[\alpha]_D$ +55.4° (*c* 4.6, MeOH),[1] by spectroscopic (^1H and ^{13}C NMR, IR, MS) and chromatographic (TLC, HPLC) comparisons.[21] Furthermore, the corresponding methyl ester exhibited spectroscopic properties (^1H and ^{13}C NMR) and chromatographic behavior (TLC) in accord with those of the authentic sample prepared from natural conagenin.



Scheme 3. (a) EtN=C=N$(CH_2)_3NMe_2$·HCl, 1-hydroxybenzotriazole, 4-methylmorpholine, DMF; (b) 1 M K_2CO_3-MeOH (1:3).

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(21) Both natural and synthetic specimens were purified by HPLC (ODS column, 5% aq. MeCN) under the same conditions before spectroscopic comparisons.

(22) Selected ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) data are following. **5**: δ 8.23 (br, 1H), 5.14 (d, 1H, *J* = 3.7 Hz), 4.96 (quint, 1H, *J* = 6.4 Hz), 2.29 (*J* = 3.7, 6.4, 7.1 Hz, 1H,), 2.15 (s, 3H), 2.04 (s, 3H), 1.24 (d, 3H, *J* = 6.4 Hz), 1.08 (d, 3H, *J* = 7.0 Hz); δ 174.1, 170.9, 170.5, 72.5, 71.3, 39.4, 21.0, 20.5, 17.3, 10.4. **12**: δ 7.35 (m, 5H), 5.28 (s, 1H), 5.23 (d, 1H, *J* = 12.2 Hz), 5.18 (d, 1H, *J* = 12.2 Hz), 4.00 (dd, 1H, *J* = 5.8, 11.3 Hz), 3.80 (dd, 1H, *J* = 7.8, 11.3 Hz), 3.20 (br s, 1H), 1.48 (s, 3H), 1.42 (s, 9H); δ 173.3, 155.4, 135.4, 128.6, 128.3, 128.1, 80.3, 67.4, 67.0, 61.0, 28.3, 20.8. **13**: δ 7.35 (m, 5H), 5.24 (d, 1H, *J* = 12.4 Hz), 5.18 (d, 1H, *J* = 12.4 Hz), 5.01 (d, 1H, *J* = 5.3 Hz), 4.97 (dq, 1H, *J* = 5.0, 6.4 Hz), 4.14 (br d, 1H, *J* = 11.3 Hz), 3.86 (br d, 1H, *J* = 11.3 Hz), 3.27 (br s, 1H), 2.50 (m, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 1.56 (s, 3H), 1.20 (d, 3H, *J* = 6.4 Hz), 0.99 (d, 3H, *J* = 7.1 Hz); δ 172.7, 170.9, 170.4, 168.9, 135.1, 128.6, 128.4, 128.0, 75.0, 71.1, 67.7, 65.3, 62.3, 39.7, 21.2, 20.7, 19.6, 17.9, 9.6. Conagenin methyl ester: δ 7.56 (br s, 1H), 4.50 (br s, 1H), 4.32 (d, 1H, *J* = 2.1 Hz), 4.22 (dq, 1H, *J* = 2.1, 6.4 Hz), 4.07 (d, 1H, *J* = 11.4 Hz), 3.83 (d, 1H, *J* = 11.4 Hz), 3.80 (s, 3H), 2.40 (br s, 1H), 2.06 (tq, 1H, *J* = 2.1, 7.1 Hz), 1.82 (br s, 1H), 1.55 (s, 3H), 1.24 (d, 3H, *J* = 6.4 Hz), 0.92 (d, 3H, *J* = 7.1 Hz); δ 173.4, 173.2, 76.8, 72.0, 66.6, 62.1, 53.1, 40.6, 21.8, 20.4, 5.2.

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