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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 NaBH₄ reduction. The amino acid moiety was synthesized based upon Et₂AICI catalyzed cyclization of the epoxy trichloroacetimidate prepared from (S)-methylglycidol. Condensation of both moieties and alkaline hydrolysis led to (+)-conagenin. Copyright © 1996 Elsevier Science Ltd

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-workers¹ 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 reported³ 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 (2R,3S,4R)-2,4-dihydroxy-3methylpentanoic 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



Construction of the carboxylic acid moiety relied on the sequence involving asymmetric aldol reaction⁵ 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 methanol⁸ to the reaction mixture, the resulting chelated aldol 2 was then directly reduced⁹ with NaBH₄ in the same flask to give diol 3, $[\alpha]^{24}D$ +35.5° (c 0.46, CHCl₃), and its epimer 4, $[\alpha]^{24}D$ -44.6° (c 0.40, CHCl₃), 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 analysis¹² of the MTPA esters of the corresponding 1-acetoxy-3-hydroxy derivatives.¹³ Upon sequential acetylation and RuO₄ mediated oxidative cleavage¹⁴ of the phenyl group, 3 gave carboxylic acid 5, $[\alpha]^{24}_{D}$ +1.6° (*c* 0.37, CHCl₃), in good overall yield.



Scheme 1. (a) (-)- $(Ipc)_2BOTf$, *i*-Pr₂EtN, CH₂Cl₂, -78 ~ -23 °C, then NaBH₄, MeOH, -23 °C; (b) (i) Ac₂O, Et₃N-DMAP (cat.), CH₂Cl₂, (ii) RuCl₃ (cat.), HIO₄, CH₃CN-CCl₄-H₂O (5:5:8).

Construction of the methylserine moiety¹⁵ 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% ee¹⁸ by the Katsuki-Sharpless catalytic asymmetric epoxidation¹⁹ 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 Et₂AlCl 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, CHCl₃), was obtained in 82% yield from 6 after pivaloylation. It is interesting to note that BF₃·Et₂O 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 purity¹⁸ 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, CHCl₃). Upon successive Swern oxidation, NaClO₂ oxidation, saponification, and benzylation, 10 afforded ester 11, mp 71-72 °C; $[\alpha]^{22}_{D}$ +7.3° (*c* 1.00, CHCl₃). 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, CHCl₃).



Scheme 2. L-DIPT (0.06 equiv.), $Ti(O-i-Pr)_4$ (0.05 equiv.), *t*-BuOOH (2 equiv.), CH_2Cl_2 , -20 °C; (b) CCl₃CN, DBU (0.1 equiv.), CH_2Cl_2 , -20 °C; (c) (i) Et₂AlCl (0.5 equiv.), CH_2Cl_2 , (ii) *t*-BuCOCl, Et₃N-DMAP (cat.), CH_2Cl_2 ; (d) 1 M HCl, THF, then NaHCO₃, Boc₂O; (e) (i) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, -60 to 25 °C, (ii) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, *t*-BuOH-H₂O (4:1), (iii) 2 M NaOH-EtOH (1:5), (iv) PhCH₂Br, K₂CO₃, DMF; (f) CF₃CO₂H, CH₂Cl₂.

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-hydroxybenzotriazole²⁰ in DMF to afford enantiomerically pure amide 13, $[\alpha]^{23}_{D}$ +31.4° (*c* 0.90, CHCl₃).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 NaHCO₃ 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 ¹³C NMR, IR, MS) and chromatographic (TLC, HPLC) comparisons.²¹ Furthermore, the corresponding methyl ester exhibited spectroscopic properties (¹H and ¹³C NMR) and chromatographic behavior (TLC) in accord with those of the authentic sample prepared from natural conagenin.



Scheme 3. (a) EtN=C=N(CH₂)₃NMe₂·HCl, 1-hydroxybenzotriazole, 4-methylmorpholine, DMF; (b) 1 M K₂CO₃-MeOH (1:3).

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- (22) Selected ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (75 MHz, CDCl₃) 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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