A SHORT, STEREOCONTROLLED SYNTHESIS OF (-)-DETOXININE

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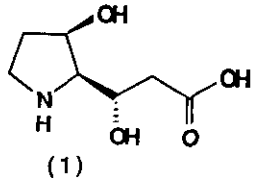
Abstract - An improved synthesis of (-)-detoxinine is described.

In 1968, a group of antagonists were found to negate the toxic side effects of blasticidin S against Bacillus cereus.[1] This group of antagonists, termed the detoxin complex, was produced by the organism Streptomyces caespitosus var. detoxicus 7072 GC1. The complex contained several components of varying detoxifying activity. Blasticidin S inhibits the virulent fungus Piriculuria oryzae which causes rice blast disease in Japan.[2] The curative effect of blasticidin S on rice plants required dosages which also caused phytotoxicity. This phytotoxicity was greatly reduced when the detoxin complex was administered with blasticidin S, without diminishing the effectiveness of the drug against Piriculuria oryzae.

Degradation of detoxin D_1 afforded the amino acids L-valine, L-phenylalanine and the previously unknown detoxinine (1).[3,4]



(1)

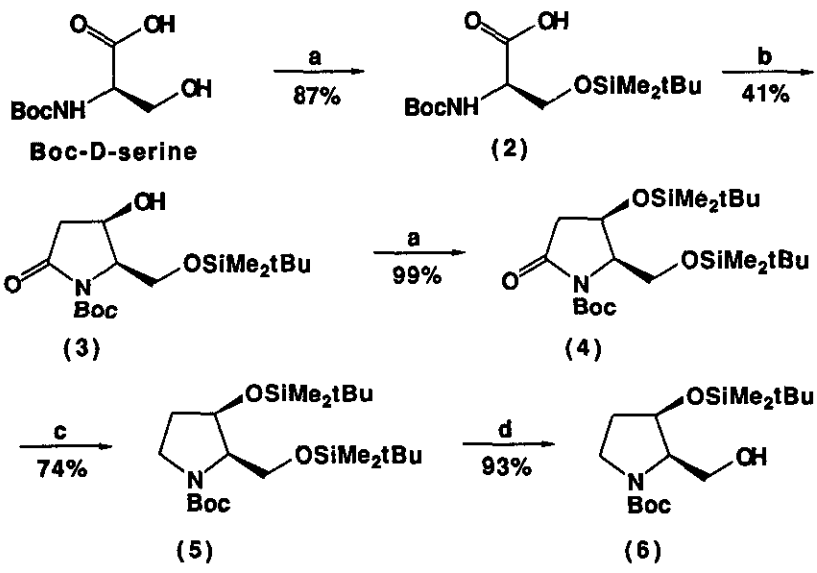
detoxinine

All detoxins contain detoxinine, except detoxins B_1 and B_2 which lack the 3-hydroxyl group in the proline ring. Detoxins B_1 and B_2 have been previously synthesized in our laboratory.[5] Detoxinine possesses several unusual structural features. Known hydroxylated amino acids may be divided into two categories. There are β-hydroxy-α-amino acids such as BMT,[6] hydroxyhomotyrosine,[7] or hydroxymethylproline,[7] in which the hydroxyl group is contained in the side chain. The other category contains such amino acids as statine,[8] dolaisoleuine,[9] or dolaproine,[9] in which the carboxylic acid group is replaced by a β-hydroxy acid unit. The amino acid detoxinine contains both of these structural features.

The synthesis of detoxinine has been reported by our group[10] as well as by other investigators.[11,12] Our original synthesis[10] represented the shortest route to this molecule.

This synthesis, however, had some shortcomings in that the proline ring system was racemic and required resolution via an aldol condensation employing a chiral enolate. Herein, we report the enantioselective synthesis of the pyrrolidine moiety, and the stereocontrolled synthesis of (-)-detoxinine. The synthesis begins with D-serine as the source of chirality (Scheme 1).

Scheme 1



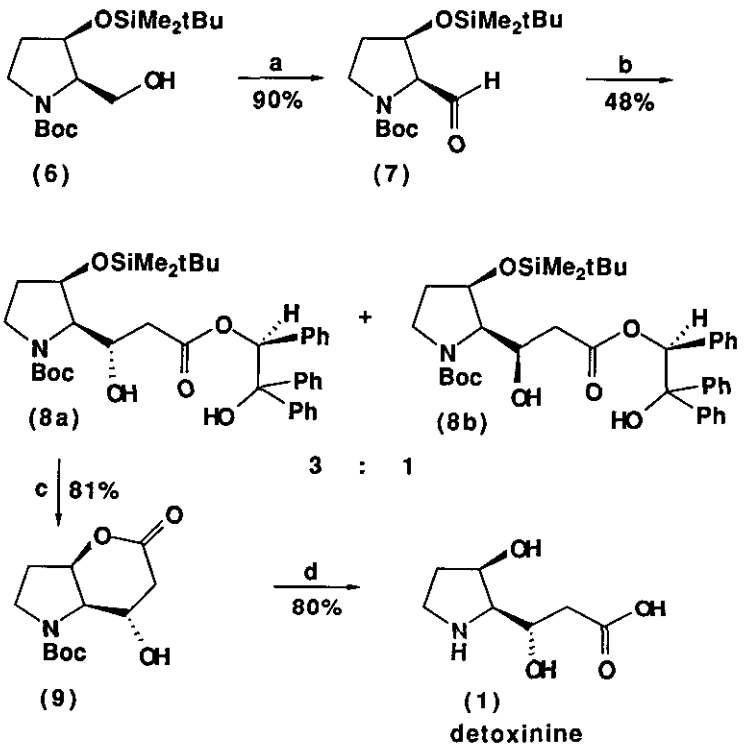
a. 1. $tBuMe_2SiCl$, DMF, Imidazole, 2. K_2CO_3; b. 1. Isopropenyl chloroformate, DMAP, Meldrum's acid, 2. EtOAc, reflux, 3. $NaBH_4$, AcOH, CH_2Cl_2; c. $BH_3{\cdot}SMe_2$, THF, reflux; d. AcOH, THF, H_2O.

D-Serine was used as its Boc derivative, followed by protection of its hydroxyl group as the TBDMS ether. This transformation gave compound 2 which was converted to pyrrolidinone 3, using Castro's method.[13] Thus, treatment of compound 2 with isopropenyl chloroformate in the presence of DMAP and Meldrum's acid, followed by heating in ethyl acetate, afforded the tetramic acid which was not isolated but treated directly with $NaBH_4$. This transformation gave the pyrrolidinone 3 in a 41% yield from compound 2, as the only diastereomer for the reaction sequence. The enantiomeric excess of compound 3 was 93% as determined by the ^1H nmr and HPLC analysis of its Mosher's ester. The secondary hydroxyl group of compound 3 was protected as its TBDMS ether in 99% yield. The reduction of Boc protected amides has been reported by Ohfune and Tomita.[14] They found that only borane-dimethyl sulfide complex gave complete reduction to the Boc protected amine. Treatment of compound 4 with $BH_3{\cdot}SMe_2$, at reflux, gave a 74% yield of the fully protected pyrrolidine 5. For the next part of the synthesis, the

primary hydroxyl group was to be oxidized to the aldehyde.

Regioselective cleavage of the silyl group on the primary alcohol was achieved using hydrolytic conditions (3:1:1; AcOH:THF:H_2O), at 0°C. A 93% yield of compound 6 was obtained. The high regiocontrol for this cleavage may be explained by assistance of the Boc carbonyl in delivering a proton to the oxygen of the primary silyl ether. The primary alcohol was then oxidized to the aldehyde using Swern conditions, with trifluoroacetic anhydride as the DMSO activator[15] (Scheme 2).

Scheme 2



a. $(CF_3CO)_2O$, DMSO, Et_3N; b. (S)-2-acetoxy-1,1,2-triphenylethanol LDA, $MgBr_2$; c. $nBu_4N^+F^-$, THF; d. TFA, Ion exchange chromatography.

Aldol condensation using the chiral enolate of (S)-2-acetoxy-1,1,2-triphenylethanol, was carried out as described by Braun and Devant.[16] This reaction gave a 3:1 mixture of diastereomeric alcohols with the major diastereomer having the correct relative configuration at the hydroxyl center. Treatment of 8a with tetrabutylammonium fluoride cleaved the silyl ether and

resulted in an intramolecular lactonization. This cyclization gave compound 9 ($[\alpha]_D^{24}$ -26.4°, ($CHCl_3$)) in 81% yield. This product was identical to the racemic compound reported by Häusler.[11] The strategy described afforded (-)-detoxinine (1) in twelve steps, in a highly *stereocontrolled manner.*

This approach also constitutes a chiral synthesis of 2-substituted 3-hydroxypyrrolidinols by extending the methodology reported by Castro.[13]

EXPERIMENTAL SECTION

GENERAL

All solvents were reagent grade. Anhydrous tetrahydrofuran (THF) was distilled from sodium/benzophenone. Anhydrous methylene chloride was distilled from calcium hydride. N,N-Dimethylformamide *(DMF) was distilled from phosphorus pentoxide. Organic bases were reagent* grade. Triethylamine and diisopropylamine were distilled from calcium hydride. Organic acids were reagent grade. Trifluoroacetic acid was distilled from phosphorous pentoxide.

Melting points were determined with a Thomas-Hoover melting point apparatus. They are expressed *in degrees centigrade (°C) and are uncorrected. Optical rotations (in degrees,* °) were measured with a Perkin-Elmer Model 241 polarimeter at the sodium D line. Proton magnetic resonance spectra (^1H-nmr) were recorded on a Bruker WM 250 MHz Fourier transform spectrometer. Chemical shifts are measured in parts per million (δ) relative to tetramethylsilane *(TMS) or deuterated chloroform as an internal standard. Coupling constants (J values) are in* Hertz (Hz). Multiplicities are designated as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), and multiplet (m). Infrared spectra (Ir) were obtained on a Perkin Elmer Model 281 B spectrometer. Absorptions are reported in wave numbers (cm^{-1}) and their intensities are designated as broad (b), strong (s), medium (m), and weak (w). *The spectra taken were* referenced to the 1601 cm^{-1} band of polystyrene, and only the most prominent or characteristic absorptions are noted. High resolution mass spectra (HRMS) were obtained on a Hitachi-Perkin Elmer RMH-2 high resolution double focusing, electron impact spectrometer or a Vacuum Generator's V.G. 7070h spectrometer interfaced with a Kratos DS-50-S data system.

Analytical thin layer chromatography (tlc) was performed on silica gel plates (0.25 cm) precoated with a fluorescent indicator. Visualization was effected with ultraviolet light, ninhydrin (3% w/v) in absolute ethanol containing 2% acetic acid, or phosphomolybdic acid reagent (7% w/v) in absolute ethanol. Column chromatography was performed on Merck silica gel 60 (230-400 mesh).

N-[(1,1-Dimethylethoxy)carbonyl]-O-[(1,1-dimethylethyl)dimethylsilyl]-D-serine (2).

To Boc-D-serine (5.83g, 28.6 mmol) in dimethylformamide (50 ml), at 0°C and under an atmosphere of argon, was added imidazole (9.8g, 144 mmol). After the solution became clear, *tert*-butyldimethylsilyl chloride (10.8g, 71.7 mmol) was added. The reaction was allowed to warm to room temperature. After 24 h, the reaction mixture was diluted with ether (250 ml) and poured into saturated NaCl (100 ml). The organic layer was separated and washed with 10% HCl (25 ml), and saturated NaCl (50 ml). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was dissolved in a solution of MeOH:THF:1M K_2CO_3 (3:1:1, 90 ml) and stirred at room temperature for 6 h. The reaction mixture was concentrated under reduced pressure to 0.25 its total volume. The remaining solution was acidified (2M $KHSO_4$) to pH 3-4 and extracted with ether (3 x 200 ml). The ether layers were combined, dried (Na_2SO_4), and concentrated under reduced pressure. Compound 2 (7.945g, 87% yield) was obtained as a foam; HRMS calcd. for $C_{14}H_{30}NO_5Si$ (M^++H): 320.1893. Found: 320.1879. Ir ($CHCl_3$): 3680 (w), 3450 (m), 1705 (s), 1620 (s), 1400 (m), 1375 (m), 1260 (m), 1170 (m), 1105 (m), 845 (s) cm^{-1}. ^{1}H-Nmr ($CDCl_3$): δ 0.07 (6H, d, J=4.2), 0.81 (9H, s), 1.43 (9H, s), 3.78-3.92 (3H, m), 5.52 (1H, bs).

(4R,5S)-5-[(1,1-Dimethylethyl)dimethylsilyl]oxymethyl-4-hydroxy-1-[(1,1-dimethylethoxy)carbonyl]-pyrrolidin-2-one (3).

To 2 (6.945g, 21.74 mmol) in dry methylene chloride (100 ml), at 0°C, and under an atmosphere of argon, was added Meldrum's acid (3.29g, 22.8 mmol) and dimethylaminopyridine (5.31g, 43.5 mmol). A solution of isopropenyl chloroformate (2.8g, 23.9 mmol) in dry methylene chloride (10 ml) was added *via* a syringe pump over 1 h. The reaction mixture was stirred at 0°C for an additional 2 h. The mixture was poured into a separatory funnel and washed with 5% $KHSO_4$ (2 x 100 ml) and water (50 ml). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The resulting crude product was dissolved in ethyl acetate (100 ml) and heated to reflux for 0.5 h. The reaction mixture was cooled and concentrated under reduced pressure. Attempts to purify this material were unsuccessful. The crude product was dissolved in dry methylene chloride (100 ml) and cooled to 0°C under an atmosphere of argon. To the reaction mixture was added $NaBH_4$ (1.50g, 39.7 mmol) portionwise over a 0.5 h period. The suspension was kept at 0°C for 4 h. The reaction mixture was poured into water (25 ml) and stirred until no solid borohydride reagent remained. The layers were separated and the organic layer was washed with water (25 ml), dried (Na_2SO_4), and concentrated under reduced pressure. The resulting crude product was purified using column chromatography (ethyl acetate: petroleum ether 20:80 to 35:65).

Pure 3 (2.9446g, 41% yield) was obtained as a white solid; mp 130-131.5°; R_f 0.32, ethyl acetate : petroleum ether (3:7); $[\alpha]_D^{22}$ -54° (c 1.15, $CHCl_3$). HRMS calcd. for $C_{16}H_{35}N_2O_5$ (M^++NH_4): 363.2315. Found 363.2265. Ir ($CHCl_3$): 3520 (m), 1795 (s), 1755 (s), 1710 (m), 1480 (w), 1380 (m), 1320 (m), 1290 (w), 1260 (m), 1155 (s), 1080 (m), 870 (m), 840 (m) cm^{-1}. ^1H-Nmr ($CDCl_3$): δ 0.06 (6H, d, J=4.8), 0.87 (9H, s), 1.50 (9H, s), 2.58 (1H, dd, J^1=8.5, J^2=17.5), 2.80 (1H, dd, J^1=8.9, J^2=17.5), 2.86-2.88 (1H, bs), 4.08 (2H, d, J=7.4), 4.17 (1H, dt, J^1=7.9, J^2=2.8), 4.47-4.57 (1H, m).

(4R,5R)-4-[(1,1-Dimethylethyl)dimethylsilyl]oxy-5-[(1,1-dimethylethyl)dimethylsilyl] oxymethyl-1-[(1,1-dimethylethoxy)carbonyl]pyrrolidin-2-one (4).

To a solution 3 (1.61g, 4.86 mmol) in dry DMF (10 ml) was added *tert*-butyldimethysilyl chloride (0.915g, 6.07 mmol) and imidazole (0.827g, 12.1 mmol). The reaction mixture was stirred for 5 h and then diluted with ether (100 ml). The mixture was washed with saturated NaCl (20 ml), 5% HCl (2 x 20 ml), 5% $NaHCO_3$ (20 ml) and saturated NaCl (20 ml). The organic layer was dried (Na_2SO_4), and concentrated under reduced pressure. The resulting crude solid was purified by column chromatography (ethyl acetate : petroleum ether, 5:95 to 10:90). Pure 4 (2.2047g, 99%) was obtained as a white solid; mp 78-79°; R_f 0.41, ethyl acetate: petroleum ether (1:9); $[\alpha]_D^{22}$ -43° (c 1.60, $CHCl_3$). HRMS calcd. for $C_{21}H_{42}NO_5Si_2$ (M-CH_3); 444.2602. Found: 444.2626. Ir ($CHCl_3$): *1795 (s), 1755 (s), 1720 (m), 1470 (w), 1360 (m), 1330 (m), 1295 (m), 1270 (m),* 1165 (s), 1100 (m), 1060 (w), 880 (s), 850 (s) cm^{-1}. ^1H-Nmr ($CDCl_3$): δ 0.01 (6H, d, J=3.6), 0.05 (6H, d, J=1.7), 0.83 (9H, s), 0.88 (9H, s), 1.50 (9H, s), 2.47 (1H, dd, J^1=10.2, J^2=16.4), 3.88-4.03 (3H, m), 4.43-4.50 (1H, m).

(2R,3R)-2-[(1,1-Dimethylethyl)dimethylsilyl]oxy-3-[(1,1-dimethylethyl)dimethylsilyl]oxymethyl-1-[(1,1-dimethylethoxy)carbonyl]pyrrolidine (5).

To a solution of 4 (1.9233g, 4.1831 mmol) in dry THF (20 ml) was added borane-dimethylsulfide (12.6 ml of a 1M solution in THF). The solution was warmed to 70°C for 3 h. The reaction mixture was cooled to room temperature, diluted with ether (200 ml), and quenched with saturated ammonium chloride (25 ml). The reaction mixture was separated. The organic layer was washed with 5% HCl (2 x 25 ml), 5% $NaHCO_3$ (25 ml), saturated NaCl (25 ml), then dried (Na_2SO_4), and concentrated under reduced pressure. The resulting crude oil was purified by column chromatography (ether : petroleum ether, 3:97 to 5:95). Pure 5 (1.371g, 74% yield) was obtained as a colorless oil; R_f 0.33, ether : petroleum ether (5:95); $[\alpha]_D^{24}$ -24° (c 1.37, $CHCl_3$). HRMS calcd. for $C_{22}H_{48}NO_4Si_2$ (M^++H): 446.3122. Found: 446.3145. Ir ($CHCl_3$): 1700 (s), 1480 (m), 1470 (m), 1420 (s), 1270 (s), 1180 (m), 1160 (m), 1140 (s), 1100 (s), 1025 (w), 1015 (w),

980 (m), 890 (s), 845 (s) cm^{-1}. ^1H-Nmr ($CDCl_3$): δ 0.01 (6H, d, J=1.8), 0.04 (6H, s), 0.85 (9H, s), 0.87 (9H, s), 1.43 (9H, s), 1.81-1.91 (1H, m), 2.03-2.14 (1H, m), 3.25-3.37 (2H, m), 3.52-3.64 (1H, m), 3.79-3.84 (2H, m), 4.22-4.39 (1H, m).

(2R,3R)-2-[(1,1-Dimethylethyl)dimethylsilyl]oxymethyl-3-hydroxymethyl-1-[(1,1-dimethylethoxy)-carbonyl]pyrrolidine (6).

To a solution of 5 (1.1719g, 2.6288 mmol) in THF (5 ml) at 0°C was added water (5 ml) and acetic acid (15 ml). Stirring at 0°C was continued for 8 h. After this time, the reaction mixture was concentrated under reduced pressure. The excess water was removed by azeotroping with toluene. The crude oil was purified by column chromatography (ethyl acetate : petroleum ether, 10:90 to 20:80). Pure 6 (0.811g, 93% yield) was obtained as an oil which solidified upon refrigeration yielding a low melting solid; R_f 0.18, ethyl acetate : petroleum ether (10:90); $[\alpha]_D^{24}$ -34.4° (c 1.99, $CHCl_3$). HRMS calcd. for $C_{16}H_{34}NO_4Si$ (M^++H): 332.2257. Found: 332.2246. Ir ($CHCl_3$): 3550 (br), 3400 (br), 1690 (s), 1420 (s), 1370 (m), 1280 (m), 1250 (s), 1100 (m), 1070 (m), 1050 (m), 1010 (w), 950 (w), 850 (s) cm^{-1}. ^1H-Nmr ($CDCl_3$): δ 0.08-0.12 (6H, d,J=6.2), 0.88 (9H, s), 1.46 (9H, s), 1.77-1.95 (2H, m), 3.37-3.47 (2H, m), 3.65-3.72 (1H, m), 3.82-3.85 (2H, m), 4.37-4.48 (2H, m).

The reactions in Scheme 2 were carried out as reported in our previous synthesis of detoxinine. For details, please see reference 10.

ACKNOWLEDGEMENTS.

Financial support from the National Institutes of Health (National Cancer Institute, grant CA 40081-02) is gratefully acknowledged. We thank Drs. Häusler and Y. Ohfune for providing us with samples of detoxinine and Drs. G. Furst and J. Dykins for their support with the high field ^1H-nmr and mass spectral data.

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Received, 11th July, 1988