# Efficient Conversion of Tomatidine into Neuritogenic Pregnane Derivative

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**Moderate acetylation of tomatidine with anhydrous acetic acid and pyridine for 20 h at r.t., followed by pseudomerization in ice-water, gave a $\delta^{20(22)}$-pseudo compound, which was then subjected to ozonolysis to provide a pregnane derivative in a total 54% yield showing neuritogenic and NGF-enhancing activities.**

**Key words** tomatidine; acetylation; $\delta^{20(22)}$-pseudo compound; ozonolysis; pregnane derivative; neuritogenic activity

The steroidal hormones are mainly derivatized from Dioscoreacea plants to produce birth controllable medicine, the pill. The raw material bearing steroidal hormones are strongly disired. The aerial parts of tomatoes are discarded after picking the fruits of tomatoes. Taking into the consideration of an effective utilization for tomato rejectings, we tried an efficient conversion of tomatidine (**1**) into a pregnane derivative, possessing the NGF-like neurite extension capability being available for dementia.

Tomatidine (**1**) is the sapogenol of tomatine which is contained in *ca.* 0.1% in the aerial parts of tomato. Effective application of the aerial parts after picking the fruits is strongly desired. Previously, Sato *et al.* reported that refluxing of tomatidine with anhydrous acetic acid produced a hydrogenable triaetate, $\delta^{20(22)}$-pseudo compound, which was subsequently oxidized with chromic acid to give 5$\alpha$-pregn-16-en-3$\beta$-ol-20-one in an overall *ca.* 27.3% yield.[1)] We have now developed a new conventional method into a pregnane derivative from tomatidine. In addition, we have revealed that the product of the pregnane derivative possesses neuritogenic and NGF-enhancing activities.

Tomatidine (**1**) was acetylated with anhydrous acetic acid and pyridine for 20 h at r.t. After the reaction mixture was poured into ice-water and left to stand overnight, the deposited white precipitate was collected by filtration. The product was purified by using silica gel column chromatography to provide a homogeneous acetate (**2**) in a 62.1% yield, whose EI-MS exhibited a molecular ion at *m*/*z* 499 corresponding to $C_{31}H_{49}NO_4$. The $^1$H-NMR (in $CDCl_3$) and 2D-NMR measurements with $^1$H–$^1$H and $^1$H–$^{13}$C correlation spectroscopy (COSY), and heteronuclear multiple-bond correlation (HMBC: $H_3$-19→C-1/C-10/C-5/C-9; $H_3$-18→C-12/C-13/C-14/C-17; $H_3$-21→C-20/C-22, $H_3$-27→C-24/C-25/C-26) enabled the assignments as follows: three tertiary methyls at $\delta$ 0.72 (3H, s, $H_3$-19), 0.76 (3H, s, $H_3$-18), 1.65 (3H, s, $H_3$-21) and one secondary methyl at $\delta$ 1.01 (3H, d, $J$=6.7 Hz, $H_3$-27), two oxygen-bearing methines at $\delta$ 4.83 (1H, m, H-3), 4.97 (1H, overlapped, H-16), and two acetyl groups at $\delta$ 2.07, 2.08 (each 3H, s). The $^{13}$C-NMR displayed total 31 carbon signals, which were composed of four methyl groups at $\delta$ 12.3 (C-18), 14.5 (C-19), 11.8 (C-21), and 17.8 (C-27), two oxygen-bearing methane carbons at $\delta$ 73.7 (C-3) and 84.5 (C-16), two acetyl carbon groups at $\delta$ 21.3, 23.4, 169.9, 170.3, two quaternary carbons at $\delta$ 35.7 (C-10), 43.8 (C-13), two olefinic carbons at $\delta$ 103.7 (C-20) and 152.4 (C-22), six methine carbons at $\delta$ 33.6 (C-25), 35.0 (C-8), 44.8 (C-5), 54.3 (C-14), 54.8 (C-9), 64.7 (C-17), and eleven methylene carbons at $\delta$ 21.5 (C-11), 23.7 (C-23), 27.9 (C-24), 28.7 (C-6), 32.3 (C-2), 32.5 (C-7), 34.4 (C-4, C-15), 36.9 (C-12), 39.9 (C-1), and 45.7 (C-26). Therefore, **2** could be estimated as $\delta^{20(22)}$-pseudo compound, 3$\beta$-hydroxy-26-amino-5$\alpha$,25*S*-furost-20(22)-ene-3,26-diacetate.

Next, to a solution of **2** in MeOH, ozone gas was introduced for 10 min at −40 °C. The solution was evaporated to give a residue, which was then refluxed with 3% KOH dioxane–water (1 : 1) and neutralized. The product was purified by silica gel column chromatography to give the pregnane derivative (**3**), which was obtained in a 87.0% yield as colorless needles, mp 203—205 °C, $[\alpha]_D$ +48.2° (EtOH). Compound **3** showed a peak due to a molecular ion at *m*/*z* 316, corresponding $C_{21}H_{32}O_2$ in the EI-MS. The $^1$H-NMR spectrum showed signals due to three tertiary methyls at $\delta$ 0.82 (3H, s, $H_3$-19), 0.94 (3H, s, $H_3$-18), 2.26 (3H, s, $H_3$-21), along with one oxygen bearing a methine proton at $\delta$ 3.85 (1H, m, H-3) and one olefinic proton at $\delta$ 6.62 (1H, dd, $J$=1.8, 3.1 Hz, H-16), of which assignments were made by the 2D-NMR measurements. The $^{13}$C-NMR signals were also assigned as follows: C-1-21, 32.5, 32.2, 70.6, 37.3, 45.5,

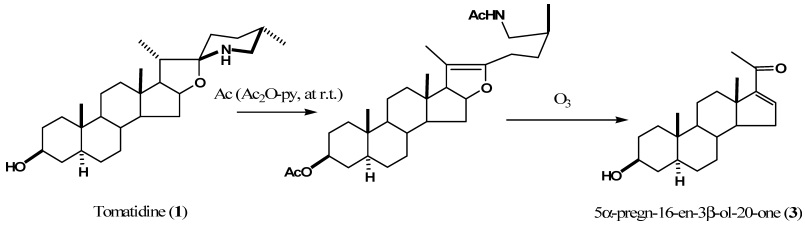


Chart 1. Preparation of 5$\alpha$-Pregn-16-en-3$\beta$-ol-20-one (**3**) from Tomatidine (**1**)

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29.1, 32.3, 34.0, 56.6, 36.0, 21.4, 39.3, 46.6, 55.1, 35.4, 144.7, 155.5, 16.3, 12.4, 196.3, 30.5, which were characterized as 5$\alpha$-pregn-16-en-3$\beta$-ol-20-one.[1,2]

This reaction procedure gave the overall yield of 54.0%, which is considerably higher yield than that of the previous Sato method. The obtained pregnane derivative (**3**) exhibited the NGF-like neurite extension induced at 5 $\mu$g/ml on PC12 cells in 2% after 1 d, 1% after 2 d, 18% after 3 d, 35% after 4 d, 46% after 5 d, 59% after 6 d, and 66% after 7 d. In control experiments, PC12 cells did not display any neurite extension in the absence of NGF, whereas NGF at 10 ng/ml had induced neurite extension in approximately 60% of the cells after 7 d. In addition, **3** was found to enhance the neuritogenic activity of NGF in an early stage. Three days after treatment, **3** at 5 $\mu$g/ml induced neurite outgrowth in 18% of PC12 cells, and a trace amount of NGF (1.5 ng/ml) showed a 6% activity, whereas this low activity of NGF was increased to 77% in the presence of **3**.

## Experimental

**Tomatidine (1) from the Aerial Parts of Tomato** The aerial part (1.25 kg) of tomato, *Lycopersicon esculentum* was refluxed with MeOH, and partitioned between hexane and MeOH. The MeOH layer was fractionated on polystyrene column chromatography eluted with $H_2O \rightarrow$MeOH. The MeOH eluate was evaporated to give an extractive (5.61 g), which was then acid-hydrolyzed, concentrated *in vacuo*, and added with water to give precipitates (3.66 g). It was then chromatographed on silica gel using with $CHCl_3$–MeOH = 100 : 1 $\rightarrow$ 60 : 1 $\rightarrow$ 40 : 1 $\rightarrow$ MeOH to provide tomatidine (185.0 mg).

An amorphous powder, EI-MS (*m*/*z*): 415 $[M]^+$. $^1$H-NMR (in $CDCl_3$) $\delta$: 0.82 (3H, s, $H_3$-18), 1.26 (3H, s, $H_3$-19), 0.86 (3H, d, $J$=6.1 Hz, $H_3$-27), 0.97 (3H, d, $J$=6.7 Hz, $H_3$-21), 3.58 (1H, m, H-3), 4.12 (1H, m, H-16). $^{13}$C-NMR (in $CDCl_3$) $\delta$: Sapogenol C-1-27, 37.1, 31.6, 71.3, 38.3, 44.9, 28.7, 32.3, 35.1, 54.5, 35.6, 21.1, 40.3, 41.0, 55.9, 32.7, 78.7, 62.1, 16.9, 12.4, 43.0, 15.8, 99.8, 26.7, 29.7, 30.9, 50.2, 19.3.

**3$\beta$-Hydroxy-26-amino-5$\alpha$,25*S*-furost-20(22)-ene-3,26-diacetate (2)** A mixture of tomatidine (**1**, 293 mg), pyridine (10 ml) and anhydrous acetic acid (5 ml) was left stand for 20 h at r.t., and then poured into ice-water. The deposited white precipitates were collected by filtration and dried. This crude acetate was subjected to silica gel chromatography eluted with $CHCl_3$ to provide 3$\beta$-hydroxy-26-amino-5$\alpha$,25*S*-furost-20(22)-ene-3,26-diacetate (**2**, 220 mg) in a yield of 62.1%.

An amorphous powder, $[\alpha]_D$ −7.5° ($c$=0.5, $CHCl_3$), EI-MS (*m*/*z*): 499 $[M]^+$, $^1$H-NMR (in pyridine-$d_5$) $\delta$: 0.72 (3H, s, $H_3$-19), 0.76 (3H, s, $H_3$-18), 1.01 (3H, d, $J$=6.7 Hz, $H_3$-27), 1.65 (3H, s, $H_3$-21), 4.83 (1H, m, H-3), 4.97 (1H, m, H-16), 2.07, 2.08 (each 3H, s, OAc). $^{13}$C-NMR (in pyridine-$d_5$) $\delta$: Sapogenol C-1-27, 39.9, 32.3, 73.7, 34.4, 44.8, 28.7, 32.5, 35.0, 54.8, 35.7, 21.5, 36.9, 43.8, 54.3, 34.4, 84.5, 64.7, 12.3, 14.5, 103.7, 11.8, 152.4, 23.7, 27.9, 33.6, 45.7, 17.8, C-OAc, 21.3, 23.4, 169.9, 170.3.

**Ozonolysis of 2 Giving 5$\alpha$-Pregn-16-en-3$\beta$-ol-20-one (3)** Diacetate (**2**, 56 mg) was dissolved in MeOH (3 ml) and cooled at −40 °C. Subsequently, ozone gas was introduced into the mixture for 10 min and the color of the reaction mixture turned blue. To the reaction mixture, 3% KOH dioxane–water (1 : 1, 3 ml) was added and refluxed for 20 min on hot water bath. After being neutralized with 1 N HCl–MeOH, the residue was chromatographed on silica gel eluting with *n*-hexane–acetone (3 : 1) to give 5$\alpha$-pregn-16-en-3$\beta$-ol-20-one (allopregnenolone) (**3**, 30.9 mg, 87.0% from **2**). Colorless needles, mp 203—205 °C, $[\alpha]_D$ +48.2° ($c$=1.0, EtOH), EI-MS (*m*/*z*): 316 $[M]^+$, $^1$H-NMR (pyridine-$d_5$) $\delta$: 0.82 (3H, s, $H_3$-19), 0.94 (3H, s, $H_3$-18), 2.26 (3H, s, $H_3$-21), 3.85 (1H, m, H-3), 6.62 (1H, dd, $J$=1.8, 3.1 Hz, H-16). $^{13}$C-NMR (pyridine-$d_5$) $\delta$: Pregnane C-1-21, 32.5, 32.2, 70.6, 37.3, 45.5, 29.1, 32.3, 34.0, 56.6, 36.0, 21.4, 39.3, 46.6, 55.1, 35.4, 144.7, 155.5, 16.3, 12.4, 196.3, 30.5.

**NGF-PC12 Cell Assay** The PC12 cells were purchased from RIKEN Cell Bank (Tsukuba, Japan). The cells were cultured in a 1.05% modified minimum essential medium Eagle (MEME) medium (ICN Biochemicals, OH, U.S.A.) supplemented with 10% fetal bovine serum, 5% horse serum, and premixed antibiotics (0.1 mg/ml streptomycin and 100 IU/ml penicillin; Invitrogen, CA, U.S.A.) under a humidified atmosphere of 5% $CO_2$ at 37 °C. The neuritogenic (induction of neurite outgrowth) activity was evaluated as follows. Twenty-thousand of PC12 cells in 1 ml of the serum-containing MEME medium were placed in each well of a 24-well microplate and precultured under the same conditions. Twenty-four hours later, the medium was replaced by 1 ml of serum-free MEME medium containing 1% DMSO and a test sample in various concentrations. For evaluation of the NGF-enhancing activity, the medium was replaced by 1 ml of serum-free MEME medium containing both 1.5 ng $\beta$-NGF (Recombinant Human NGF; R&D Systems, Minneapolis, U.S.A.) and a test sample. The morphological changes in the cells were monitored through a phase-contrast microscope every 24 h. Approximately 100 cells were counted from a randomly chosen field; this operation was repeated 3 times. The activity was represented by the percentages of PC12 cells with a neurite outgrowth longer than the cell diameter.

## References

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