Efficient Conversion of Tomatidine into Neuritogenic Pregnan 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 δ^{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, δ^{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 desired. 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 trietate, δ^{20(22)}-pseudo compound, which was subsequently oxidized with chromic acid to give 5α-pregn-16-en-3β-ol-20-one in an overall ca. 27.3% yield. 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 deposed white precipitate was collected by filtration. The product was purified by using silica gel column chromatography to give the pregnane compound (2) in a 87.0% yield as colorless needles, mp 203–205 °C, [α]_D^25 48.2° (EtOH). Compound (2) was confirmed to be a δ^{20(22)}-pseudo compound, 3β-hydroxy-26-amino-5α,25S-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 in anhydrous water (1:1) and neutralized. The product was purified by silica gel column chromatography to give the pregnane derivative (3), which was obtained in an 87.0% yield as colorless needles, mp 203–205 °C, [α]_D^25 48.2° (EtOH). Compound (3) showed a peak due to a molecular ion at m/z 316, corresponding to C_{31}H_{49}NO_4. The 1H-NMR spectrum showed signals due to three tertiary methyls at δ 0.72 (3H, s, H₃-19), 0.76 (3H, s, H₃-18), 1.65 (3H, s, H₃-21) and one secondary methyl at δ 1.01 (3H, d, J=6.7 Hz, H₃-27), two oxygen-bearing methines at δ 4.83 (1H, m, H₃-25), 4.97 (1H, overlapped, H-16), and two acetyl carbon groups at δ 2.07, 2.08 (each 3H, s). The 13C-NMR displayed total 31 carbon signals, which were composed of four methyl groups at δ 12.3 (C-18), 14.5 (C-19), 11.8 (C-21), and 17.8 (C-27), two oxygen-bearing methane carbons at δ 73.7 (C-3) and 84.5 (C-16), two acetyl carbon groups at δ 21.3, 23.4, 169.9, 170.3, two quaternary carbons at δ 35.7 (C-10), 43.8 (C-13), two olefinic carbons at δ 103.7 (C-20) and 152.4 (C-22), six methine carbons at δ 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 δ 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 δ^{20(22)}-pseudo compound, 3β-hydroxy-26-amino-5α,25S-furost-20(22)-ene-3,26-diacetate.

Chart 1. Preparation of 5α-Pregn-16-en-3β-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 5α-pregn-16-en-3β-ol-20-one.  

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 μg/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. The neuritogenic (induction of neurite outgrowth) activity was evaluated as 54.0% 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 of tomato, Solanum esculentum, was refluxed with MeOH, and partitioned between hexane and MeOH. The MeOH layer was fractionated on polystyrene column chromatography eluted with H2O→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 CHCl3–MeOH (1 : 1, 3 ml) was added and refluxed for 20 min on hot water bath. The colorless needles, mp 203—205 °C, [α]D25 +48.2° (c=1.0, EtOH), EI-MS (m/z): 316 [M]+. 1H-NMR (pyridine-d5): 0.82 (3H, s, H3-19), 0.94 (3H, s, H3-18), 2.26 (3H, s, H3-21), 3.85 (1H, m, H-20), 6.02 (1H, dd, J=1.8, 3.1 Hz), 1H, H-16). 13C-NMR (pyridine-d5): 31.6, 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, 16.4, 12.4, 196.3, 30.5.

Ozonolysis of 2 Giving 5α-Pregn-16-en-3β-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 1N HCl–MeOH, the residue was chromatographed on silica gel eluting with n-hexane-acetone (3 : 1) to give 5α-pregn-16-en-3β-ol-20-one (allopregnenolone) (3, 3.09 mg, 87.0% from 2). Colorless needles, mp 203—205 °C, [α]D25 +48.2° (c=1.0, EtOH), EI-MS (m/z): 316 [M]+. 1H-NMR (pyridine-d5): 0.82 (3H, s, H3-19), 0.94 (3H, s, H3-18), 2.26 (3H, s, H3-21), 3.85 (1H, m, H-20), 6.02 (1H, dd, J=1.8, 3.1 Hz), 1H, H-16). 13C-NMR (pyridine-d5): 31.6, 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, 16.4, 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% CO2 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 β-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