Activated Charcoal-mediated Hydroxylation under Very Mild Conditions: Conversion of Physalin B into 25-Hydroxyphysalin B

Bunsho Makino,[a] Masao Kawai,*[a] Tatsuo Yamamoto,[a] Hatsuo Yamamura,[a] Yasuo Butsugan,[a] Mitsuo Hayashi[b] and Kiyoshi Ogawa[b]

[a] *Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466, Japan*
[b] *Institute for Life Science Research, Asahi Chemical Industry Co. Ltd., Ohito-cho, Shizuoka, 410-23, Japan*

Activated charcoal treatment of physalin B, a 13,14-seco-16,24-cyclosteroidal constituent of *Physalis alkekengi*, in MeOH at room temperature yields a product possessing a hydroxy group at the α-position of the δ-lactone carbonyl function, namely 25-hydroxyphysalin B, in high yield.

Activated charcoal is commonly used for the purpose of removing coloured impurities and/or separating desired compounds in natural product chemistry. Besides its use as a solid support for various catalysts, activated charcoal alone can also catalyse reactions of organic compounds,[1] leading to undesired artifacts in natural product purification.

In this communication, a novel activated charcoal-mediated hydroxylation reaction found during the isolation of highly oxygenated steroidal constituents is described.

Physalins are the steroidal constituents of *Physalis* plants (*Solanaceae*) possessing a novel 13,14-seco-16,24-cycloergostane skeleton. From the epigeal part of *P. alkekengi* var. *francheti* (Japanese name: Hozuki) physalin A,[2] physalin B,[2] physalin C,[3] physalin L,[4] physalin M,[5] physalin N[6] and physalin O[6] have been isolated and characterised. In the course of our studies directed towards the isolation of new physalins from this plant, a $CHCl_3$ extract containing physalin B **1** was 'purified' on an activated-charcoal column, which was eluted with acetone–H_2O or acetone–MeOH. Silica gel TLC of the eluate unexpectedly indicated the presence of a new component and also loss of **1**, suggesting that **1** had been converted into an unknown product. Consequently, study upon the nature of this reaction including the structural determination of the newly formed compound has been undertaken.

Activated charcoal (Nacalai Tesque Inc., No. 079-12, for column chromatographic use, 60–150 mesh) was added to a MeOH solution of **1** and the mixture was stirred at room temp. The reaction was monitored by silica gel TLC, which indicated the decrease of **1** with concomitant increase of the new compound **2**. Addition of ammonium acetate to the mixture was found to accelerate the conversion. In a typical run, **1** (164 mg), activated charcoal (500 mg), and ammonium acetate (84 mg) in MeOH (80 ml) were stirred at room temp. After 17.5 h the conversion of **1** into **2** was complete as checked by TLC and the product was chromatographed over silica gel using $CHCl_3$ as eluent yielding **2** (112 mg), m.p. 216–218 °C (colourless prisms from $CHCl_3$).

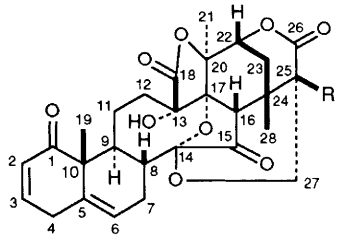
Table 1 ^{13}C NMR spectral data of 25-hydroxyphysalin B **2** compared with physalin B **1**[a] measured in [$^2H_6$]DMSO solution

Carbon	δ	Carbon	δ	Carbon	δ
1	202.4 (0.0)	11	24.2 (+0.1)	21	21.9 (+0.2)
2	126.9 (0.0)	12	25.5 (−0.1)	22	76.6 (+0.3)
3	146.1 (0.0)	13	80.4 (−0.3)	23	28.1 (−3.3)
4	32.3 (0.0)	14	106.1 (−0.2)	24	35.5 (+5.0)
5	135.6 (+0.1)	15	209.2 (−0.1)	25	73.6 (+24.2)
6	123.3 (−0.1)	16	54.1 (−0.1)	26	168.4 (+1.2)
7	24.3 (−0.1)	17	78.2 (0.0)	27	64.5 (+3.9)
8	39.7 (−0.5)	18	171.6 (−0.2)	28	18.9 (−5.5)
9	33.2 (+0.1)	19	16.7 (−0.1)		
10	51.9 (0.0)	20	79.5 (−0.8)		

[a] Chemical shift differences between **2** and **1** are given in parentheses. Positive and negative values indicate that the carbon signal in **2** resonates at lower and higher field, respectively, than the corresponding signal in **1**.

The product **2**, $[\alpha]_D^{25}$ −132 (*c* 0.19, acetone), showed a molecular ion peak at *m/z* 526 on electron-impact mass spectra. High resolution mass spectrometry established its molecular formula as $C_{28}H_{30}O_{10}$ indicating an increment of one oxygen atom compared with that of parent **1**. The 400 MHz ^1H NMR spectra of **2** taken in [$^2H_6$]acetone solution showed signals of three alkenic protons at δ 5.86 (dd, *J* 10 and 2 Hz, H-2), 6.90 (ddd, *J* 10, 5, and 3 Hz, H-3), and 5.62 (br d, *J* 6 Hz, H-6) due to the 2,5-dien-1-one system of the A/B ring moiety, which was shown to be intact during the conversion of **1** into **2**. A marked difference between the ^1H NMR spectra of **1** and **2** was the absence of the methine proton signal at C-25 in **2** and the presence of an additional tertiary OH signal at δ 5.34 in **2**, suggesting the new compound **2** to be a 25-hydroxylated derivative of **1**. Spin multiplicity of the proton signals assignable to C-27 methylene group (δ 3.59, d, *J* 13 Hz and 4.11, d, *J* 13 Hz) lacking vicinal coupling also supported the presence of an OH group at C-25 position. The ^{13}C NMR spectral data of **2** measured in [$^2H_6$]DMSO solution are summarized in Table 1 in comparison with those of **1**.[6] The C-25 signal of **2** was observed at much lower field (δ 73.6) than the corresponding signal of **1** (δ 49.4) indicating the hydroxylation at this position. Significant chemical shift differences were observed between the corresponding carbons of **1** and **2** in the vicinity of C-25, *i.e.* differences were larger than 1 ppm for C-23 to C-28. Other carbon resonances, however, did not show any significant differences between **1** and **2** indicating that no skeletal rearrangement occurred during the conversion of **1** into **2**. The structure of **2** as 25-hydroxyphysalin B was also confirmed by detailed 400 MHz ^1H NMR spectral analysis.†

In order to examine whether this novel hydroxylation is specific to a particular source of activated charcoal, the reactions using differently treated Norit were carried out for comparison. All the charcoal examined were found to mediate



1 R = H **2** R = OH

† 400 MHz ^1H NMR data of **2** in [$^2H_6$]acetone are as follows: δ 1.22 (s, CH_3-19), ~1.25 (m, H-11β), 1.31 (s, CH_3-28), 1.62 (dd, $J_{12\beta,12\alpha}$ 16 Hz, $J_{12\beta,11\beta}$ 10 Hz, H-12β), 1.91 (s, CH_3-21), ~1.9 (m, H-23*S*), ~2.1 (m, H-7α, H-8 and H-11α), ~2.3 (m, H-7β and H-12α), 2.53 (dd, $J_{23R,23S}$ 15 Hz, $J_{23R,22}$ 3 Hz, H-23R), 2.87 (s, H-16), ~2.95 (m, H-4α and H-9), 3.37 (br d, $J_{4\beta,4\alpha}$ 21 Hz, H-4β), 3.59 (d, $J_{27R,27S}$ 13 Hz, H-27*R*), 4.11 (d, $J_{27S,27R}$ 13 Hz, H-27*S*), 4.59 (dd, $J_{22,23R}$ 3 Hz, $J_{22,23S}$ 2 Hz, H-22), 4.93 (s, HO-13), 5.34 (s, HO-25), 5.62 (d, $J_{6,7\beta}$ 6 Hz, H-6), 5.86 (dd, $J_{2,3}$ 10 Hz, $J_{2,4\beta}$ 2 Hz, H-2), and 6.90 (ddd, $J_{3,2}$ 10 Hz, $J_{3,4\alpha}$ 5 Hz and $J_{3,4\beta}$ 3 Hz, H-3).

the reaction of **1** into **2**, and the reaction rate was in the order: Norit EXW (acid washed with HCl) > Norit A (untreated) > activated charcoal for chromatography (Nacalai Tesque Inc.) > Norit I (neutralized). Since no oxidizing agent was added to the reaction mixture, oxygen molecules or atoms adsorbed chemically or physically on the activated charcoal were assumed to be responsible for the unexpected hydroxylation of **1**. In fact, when activated charcoal treated with aqueous Na_2SO_3 solution was used instead of the charcoal equilibrated with atmospheric oxygen, no production of **2** was observed. Activated charcoal-catalysed oxidation of cyclohexene to yield cyclohexenone and cyclohexenol was reported,[7] but the hydroxylation described in this communication is noteworthy considering the high yield and unusually mild conditions. Autoxidation of carbonyl compounds to give α-hydroxylated products is known as a similar type of reaction but strongly alkaline conditions are required to produce carbanion which reacts with oxygen molecule.[8] Although the conversion of **1** into **2** proceeds under very mild conditions, the reaction mechanism may involve proton abstraction from the C-25 position since addition of triethylamine accelerates the hydroxylation markedly. The presence of acetic acid, on the other hand, served to slow down the rate of the reaction, and the reaction rate in the presence of additives is in the order: $Et_3N \gg AcONH_4 >$ (none) $\gg$ AcOH. Preliminary experiments have indicated that other physalins also yield similar products to **2** possessing a hydroxy group at the C-25 position, and studies on the nature of this hydroxylation reaction and its applicability to other type of compounds are being undertaken. Although the details of this activated charcoal-mediated hydroxylation remain unexplained, the results described here demonstrate that deliberate care must be taken when applying activated charcoal to the isolation of complicated organic compounds.

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