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Synthesis of Stereoisomeric Triterpene Alcohols by the Reduction of Hydroxydammarenone-Ⅱ

(Research note)

by

Ito, Yukiko[1], Hayashi, Yoshioki[2], Kato, Atsushi[3] and Ohara, Seiji[4]

Summary : Hydroxydammarenone-Ⅱ which has a carbonyl group in its molecule, was reduced on several conditions to obtain two types of alcohols(stereoisomers ; α , β). Hydroxydammarenone-Ⅱ was isolated from the benzene extract of the wood of Keruing(tropical wood, *Dipterocarpus* sp.) by column chromatography and recrystalization. The reduction with LiBH(*sec*-Bu)$_3$ gave two types of alcohols, and a ratio of α and β alcohols was different according to the reaction temperature. At higher temperature, α alcohol was formed more. High performance liquid chromatography(HPLC) with octadecylsilane(ODS) column using a solvent system of methanol and water(9:1) gave good separation of two types of alcohols. The assignment of the ^{13}C-NMR spectrum of β alcohol was revised by the modern NMR techniques.

1. Introduction

In *Panax ginseng* C. A. Meyer, one of the most famous traditional medicines, biologically active compounds called saponins are contained therein. Dammarane type triterpenes are typical sapogenins which constitute saponins in *P. ginseng*.

It is well known that biological activities are different between stereoisomers(Kijima, 1966). The wood of Keruing (tropical wood, *Dipterocarpus* sp.) contains abundantly hydroxydammarenone-Ⅱ (1)(Fig.1), one of dammarane type triterpenes. As it is a triterpene having carbonyl group at the 3 position, it is expected to be reduced to form two stereoisomer alcohols, α alcohol(2) and β alcohol(3) (Fig.1). β Alcohol(3) is formed more than α alcohol (2), because α alcohol is thermodynamically more unstable by steric repulsion(Fig.1). Almost all of naturally occurring triterpenes with hydroxyl group at 3-position are β alcohls.

In this study, two kinds of reductants, sodium borohydride($NaBH_4$) and lithium tri-*sec*-buthylborohydride (LiBH(*sec*-Bu)$_3$), were used. Sodium borohydride $NaBH_4$ is handled easily and it is used frequently. Lithium tri-*sec*-buryl borohydride LiBH(*sec*-Bu)$_3$ is bulky, so it is known as selective reducing reagent, especially for cyclohexanone(Brown, 1970). The separation of the reduction products is also discussed.

2. Experimental

2.1 General Methods

Mass spectra(MS) by electron impact method were obtained with a JEOL JMS-DX303 mass spectrometer, infrared spectra(IR) with a JASCO IRA-2 spectrometer, and nuclear magnetic resonance spectra(NMR) with a JEOL JMS GSX-400 spectrometer using tetramethylsilane as an internal standard.

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(1)(3)(4) Wood Chemistry Division
(2) Research Coordination Division

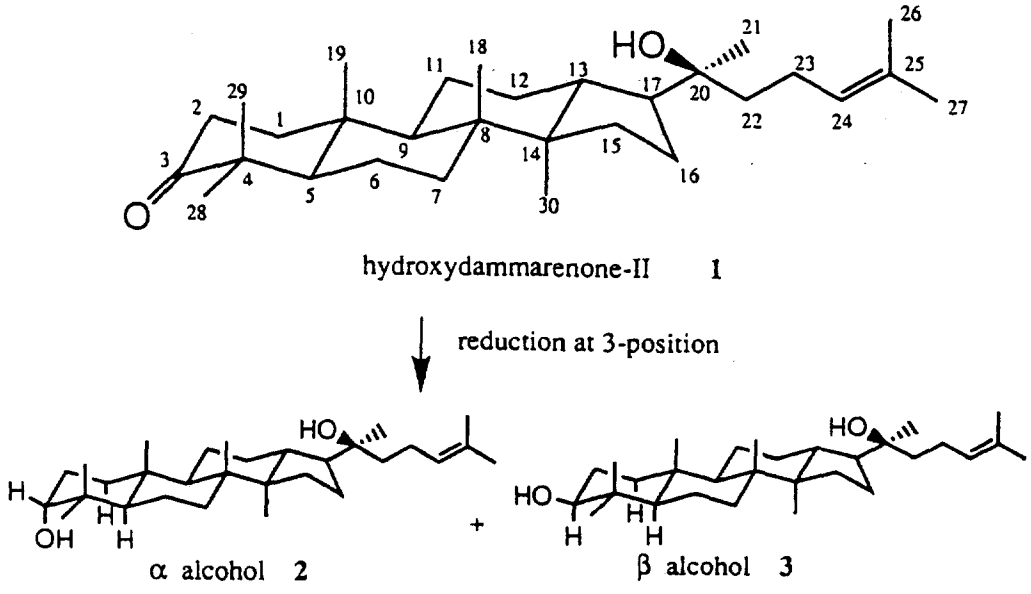

Fig. 1　Hydroxydammarenone-II(1) and its corresponding alcohols (α (2) and β (3))

Tetrahydrofurane(THF) used in the reduction was dried with calcium hydride and then distilled in the presence of sodium and benzophenone.

2.2 Plant material

Dipterocarpus sp. was collected in Kalimantan, Indonesia in 1965. Details were referred to No.206 of this bulletin(Wood technology div., 1967).

2.3 Extraction and isolation of hydroxydammarenone- Ⅱ (1)

Wood powder(9.5kg) of *Dipterocarupos* sp. was extracted with benzene(54 l) using a Soxhlet extractor to give 310g of extractives. The extractives was applied to a silica gel column chromatography(70-230mesh; MERCK Co. Ltd.) eluted with hexane/acetone(100/0-5/1) two times, followed by a fine silica gel column chromatography (230-400mesh; MERCK Co. Ltd.) eluted with hexane/acetone(100/0-10/1), to obtain a white solid. Hydroxydammarenone- Ⅱ (1) was isolated from this solid by recrystalization from warm methanol and water (28.82g, 0.3% of wood powder, colorless needles).

2.4 Reduction of hydroxydammarenone- Ⅱ (1)

2.4.1 Reduction with $NaBH_4$

To hydroxydammarenone- Ⅱ (1)(22.9mg) in isopropyl alcohol(4ml), $NaBH_4$ was added gradually at room temperature(20℃) to reduce it completely. The reaction mixture was extracted with diethyl ether, and purified by a silica gel column chromatography(230-400mesh; MERCK Co. Ltd.) eluted with hexane/acetone(100/0 - 5/1).

2.4.2 Reduction with $LiBH(sec\text{-}Bu)_3$

To hydroxydammarenone- Ⅱ (1)(114mg) in dry THF(4ml) kept at -78℃ to 50℃(Table 1), two equivalents of $LiBH(sec\text{-}Bu)_3$ were added as 1M-THF solution(0.515ml). The reaction mixture was treated with 140ml of 3M sodium hydroxide, followed by 140ml of 30% hydrogen peroxide to oxidize the organoborane. Potassium carbonate was added to saturate the aqueous phase, and then the mixture was extracted with diethyl ether. The ether extracts were purified by the same procedure as described above.

2.4.3 Quantitative analysis of reduction products by ^{1}H-NMR

The ratio of α alcohol(2) and β alcohol(3) formed by reduction was estimated by the relative intensities of the resonances due to 3-H(protons at the 3-position) in the ^{1}H-NMR spectrum.

3. Results and Discussion

3.1 Reduction with $NaBH_4$

Eight equivalents of $NaBH_4$ were necessary to reduce hydroxydammarenone-II (1)(22.9mg) to yield 20.1mg of alcohol 87% of (1). On the basis of ^{1}H and ^{13}C NMR spectra of the reaction product, it was found that only β alcohol(3) was stereoselectively produced(Table 1).

3.2 Reduction with LiBH(*sec*-Bu)$_3$

Hydroxydammarenone-II (1)(114mg) in THF was reduced immediately by two equivalents of LiBH(*sec*-Bu)$_3$ at -78℃ to 50℃ to yield 105mg of alcohol [(92% of (1))]. On the basis of ^{1}H and ^{13}C NMR spectra of the reaction product, it was found that both α (2) and β (3) alcohols were produced and a ratio of α (2) and β (3) formed was different according to the reaction temperatures(Table 1). At higher temperature, α alcohol(2) was formed more. Because of the danger of this reductant at higher temperature, the reduction was carried out at room temperature to isolate α alcohol.

3.3 Separation of α alcohol(2) and β alcohol(3)

The reduction products of hydroxydammarenone-II [((1), 2.06g)] with LiBH(*sec*-Bu)$_3$ at 24℃ were applied to a silica gel column chromatography(230 - 400mesh, 90g) eluted with hexane/acetone(100/0-90/10), and fractionated(16ml per each fraction). Fractions 1-2 were combined, and colorless needles were obtained by recrystalization from this eluent. They were identified as α alcohol(2) on the basis of ^{1}H and ^{13}C NMR data.

The high performance liquid chromatography(HPLC) system using octadecylsilane(ODS) eluted with methanol and water(9:1) showed good separation of the mixture(Fractions 3-32) of α and β alcohols(Fig. 2).

3.4 Spectral data of α alcohol(2) and β alcohol(3)

α Alcohol(2). IR : ν max(KBr) ; 3 350(broad), 2 950 cm^{-1}. MS : m/z 426(M^+-H_2O). ^{1}H-NMR : (cf : s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, tqq=triplet quartet quartet) : 5.12(1H, tqq,J=7.08, 1.46, 0.98 ; 24-H), 3.39(1H, t, J=2.93 ; 3-H), 2.1-1.0(26H, m ; methylene), 1.69(3H, d, J=0.98 ; 26-Me), 1.62(3H, s ; 27-Me), 1.14(3H, s ; 21-Me), 0.96(3H, s ; 30-Me), 0.94(3H, s ; 28-Me), 0.90(3H, s ; 18-Me), 0.86(3H, s ; 19-Me), 0.84

Table 1. The ratio of α alcohol(2) and β alcohol(3) formed by reduction of hydroxydamarenone-II (1)

Reducing reagents	Temperatures (℃)	α alcohol(2) : β alcohol(3)
$NaBH_4$	20	1 : 12.7
LiBH(*sec*-Bu)$_3$	-78	1 : 2.72
	0	1 : 1.12
	24	1 : 1.04
	40	1 : 0.93
	50	1 : 0.91

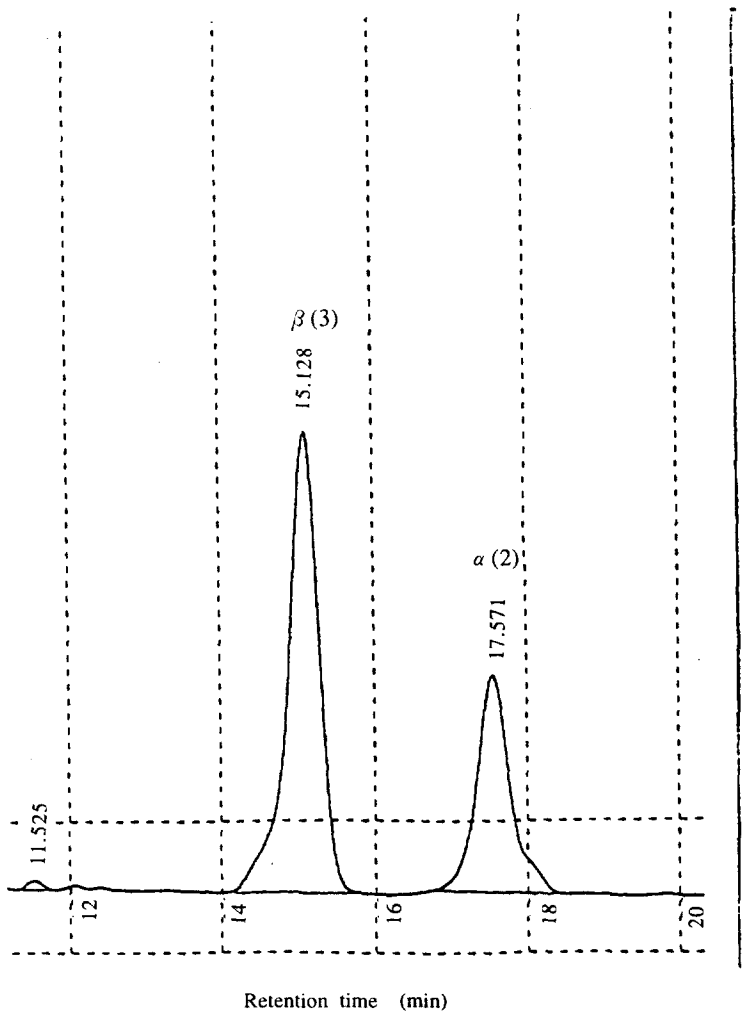

Fig. 2　HPLC chromatogram of separation of α alcohol(2) and β alcohol(3)

(3H, s ; 29-Me). ^{13}C-NMR : 131.7(25-C), 124.8(24-C), 76.4(3-C), 75.5(20-C), 50.6(14-C), 50.5(9-C), 49.9(17-C), 49.6(5-C), 42.3(13-C), 40.7(22-C), 40.6(8-C), 37.7(4-C), 37.4(10-C), 35.2(7-C), 33.7(1-C), 31.2(15-C), 28.4(28-C), 27.7(16-C), 25.8(26-C), 25.5(2-C), 25.5(21-C), 24.9(12-C), 22.7(23-C), 22.2(29-C), 21.5(11-C), 18.3(6-C), 17.8(27-C), 16.6(18-C), 16.1(19-C), 15.6(30-C).

β Alcohol(3). IR : ν max(KBr) ; 3 430(broad), 2 950 cm^{-1}. MS : m/z 426(M^+-H_2O). ^{1}H-NMR : (cf : tm=triplet and multiplet, dd=double doublet) : 5.12(1H, tm, J=7.08 ; 24-H), 3.20(1H,dd, J=11.23, 5.12 ; 3-H), 2.1-1.0(26H, m ; methylene), 1.69(3H, s ; 26-Me), 1.62(3H, s ; 27-Me), 1.14(3H, s ; 21-Me), 0.98(3H, s ; 28-Me), 0.96(3H, s ; 30-Me), 0.88(3H, s ; 18-Me), 0.85(3H, s ; 19-Me), 0.78(3H, s ; 19-Me). ^{13}C-NMR : 131.6(25-C), 124.7(24-C), 79.0(3-C), 75.4(20-C), 55.9(5-C), 50.7(9-C), 50.3(14-C), 49.9(17-C), 42.3(13-C), 40.5(22-C), 40.4(8-C), 39.1(1-C), 39.0(4-C), 37.1(10-C), 35.3(7-C), 31.2(15-C), 28.0(28-C), 27.6(16-C), 27.4(2-C), 25.7(26-C), 25.4(21-C), 24.8(12-C), 22.6(23-C), 21.6(11-C), 18.3(6-C), 17.7(27-C), 16.5(18-C), 16.2(19-C), 15.5(30-

C), 15.4(29-C).

Chemical shift data of the carbons of α alcohol(2) and β alcohol(3) were referred to those of ASAKAWA (1977). In addition, the ^{1}H-^{13}C COSY(H/C shift correlation spectroscopy) and COLOC(correlation spectroscopy via long-range coupling) spectra provided the evidence needed for assignment of olefine, C-OH, and methyl carbons. Furthermore, comparison of chemical shifts between α and β alcohols permitted assignment of the carbon signals of β alcohol.

Thirty signals were observed in the ^{13}C-NMR spectrum of β alcohol(3). The signal at 25.4ppm was assigned to 21-C(carbon at the 21-position) by consideration of ^{1}H-^{13}C COSY spectrum, which clearly showed cross peak between 21-C carbon and methyl protons at the 21-position at 1.14ppm. Furthermore, the DEPT(distortionless enhancement by polalization transfer) experiment made clear that the signal at 24.7ppm was due to methylene carbon. Consequently, the signal could be assigned to 12-C. In ASAKAWA's paper, it was reported that the signal at 25.4ppm was due to 12-C and the signal at 24.7ppm was due to 21-C. Therefore, his assignments of 21-C and 12-C should be revised.

The chemical shifts for 3-C and 29-C of β alcohol(3) differed remarkably from those of α alcohol(2). In the spectrum of β alcohol(3), 3-C and 29-C appeared at 79.0 and 15.4ppm, respectively. On the other hand, the corresponding signals of α alcohol(2) were observed at 76.4 and 22.2ppm, respectively.

In the ^{1}H-NMR spectrum of the mixture of α alcohol(2) and β alcohol(3), two signals due to 3-H were observed(Fig.3). Coupling constants ($J_{2axial(ax)\text{-}3}$ and $J_{2equatorial(eq)\text{-}3}$) of α alcohol(2) were almost equal to each other(2.93 Hz), therefore this signal(due to 3-H) looked like triplet. As the 3-H of β alcohol(3) is axial, $J_{2ax\text{-}3}$ and $J_{2eq\text{-}3}$ were quite different(11.23 and 5.12 Hz, respectively). Therefore, the signal due to 3-H was clearly double doublet.

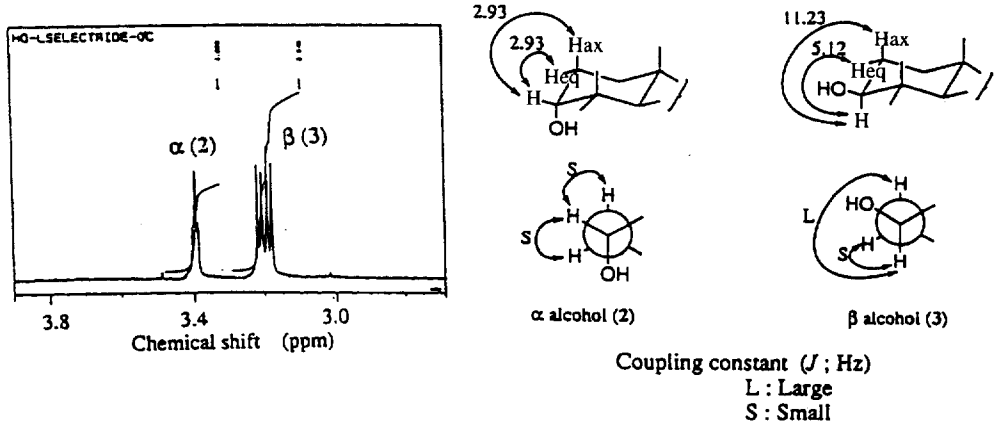

Fig. 3 ^{1}H-NMR spectrum of α alcohol(2) and β alcohol (3) at the 3-position and coupling constants (J)

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(研究資料)

ヒドロキシダマレノンーⅡの還元による立体異性トリテルペンアルコールの合成

伊藤由紀子(1)，林　良興(2)，加藤　厚(3)，大原　誠資(4)

摘　要

化合物の生理活性が化学構造上の立体異性体間で大きく異なる例は数多く知られている。クルイン材に多く含まれる，ダマラン系トリテルペンであり，カルボニル基を持つヒドロキシダマレノンーⅡは，還元反応によって2種の立体異性体アルコール，α体，β体の生成が可能である。本研究ではこれら2種の立体異性トリテルペンアルコールを得るため，還元条件及び分離精製条件を検討した。

ヒドロキシダマレノンーⅡはカリマンタン産クルイン材のベンゼン抽出物から，カラムクロマトグラフィー及び再結晶により単離精製した（無色針状晶）。水素化ホウ素ナトリウムによる還元では，ほぼ立体選択的にβ体が生成したが，$LiBH(sec\text{-}Bu)_3$を用いたテトラヒドロフラン中での還元ではα体，β体両異性体が生成した。生成比は反応温度によって異なり，反応温度を高くするほどα体の生成量が増加した。両異性体の分離は逆相系のＯＤＳカラム及びメタノール：水＝９：１の溶媒系を用いた高速液体クロマトグラフィーによって行い，分取した両異性体の各種スペクトルを測定し，分子構造を確認した。

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(1)(3)(4)　木材化工部
(2)　企画調整部