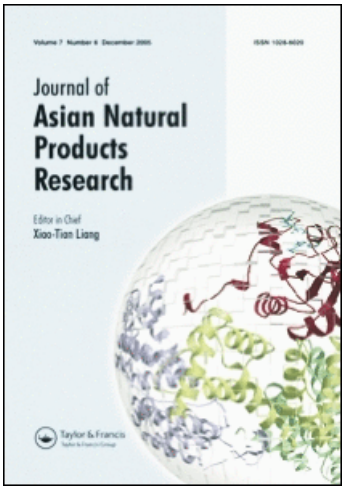

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Nimbolide and isonimbolide

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Nimbolide **1**, a potent molecule of biological significance, was isolated. Attempts were made to cleave the ether linkage in nimbolide using boron trifluoride etherate in the presence of tetrabutyl ammonium bromide so as to generate a ring-opened structure akin to azadirachtins, which are known to possess excellent antifeedant properties. However, a novel rearranged product was envisaged during the course of the reaction, which was determined as isonimbolide **2**—a structural isomer of nimbolide through spectroscopic methods.

Keywords: Nimbolide; isonimbolide; $BF_3.OEt_2$-$(C_4H_9)_4NBr$

1. Introduction

Several reports have been made on the isolation of nimbolide [1], a major tetranortriterpenoid from neem leaves. The scope of nimbolide for medicinal applications is enormous as it exhibits a wide range of activities like cytotoxicity [2], antimalarial [3], antitumour [4], antibacterial [5], etc. Neem leaf extracts containing nimbolide as the major constituent were found to posses antifeedant activity against two coleopteran pests, one of which is not controlled even by azadirachtin A [6]. However, inspite of the fact that both nimbolide and azadirachtin [7] belong to the same group of limonoids, viz., C-seco limonoids, the latter exhibits threefold more antifeedant activity than the other members of the group. The pronounced activity of azadirachtin A is attributed to the presence of free rotation of the hydroxy tricyclic hydro furan acetal fragment attached to C8. Hence to explore the semi-synthetic modifications of nimbolide to further enhance its bioactivity and to arrive at the structure activity relationship, cleavage of the ether linkage between C7 and C15 to produce a molecule akin to azadirachtins was attempted using several reagents. We report the results of our findings.

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2. Results and discussion

The C-seco limonoids except the azadirachtins are characterized by the presence of a per hydro linear naphthofuran ring. We attempted to cleave the ether linkage between C7–C15 [8] to enable free rotation about the C8–C14 bond thereby mimicking azadirachtin A. Reagents like HBr, $ZrCl_4$ resulted in uncontrolled reactions wherein $BF_3.OEt_2$-$(C_4H_9)_4NBr$ yielded a novel rearranged product **2**. The structure was ascertained using NMR (1D and 2D), mass spectra and HPLC analyses. Nimbolide eluted at a retention time of 20.76 min while the product eluted at 23.84 min. The absence of peaks with 1:1 intensity at *m/z* 79 and 81 in the mass spectrum of **2** confirmed the absence of bromine atom. A comparison of the relevant ^{13}CNMR chemical shifts of **2** and **1** is presented in table 1. These results suggest the following structure for **2** (scheme 1), which is simply the isomer of **1** and hence could be called isonimbolide.

In order to understand the mechanism of the reaction a blank reaction was performed with only $BF_3.OEt_2$ and the reaction was monitored by HPLC. The reaction resulted in the recovery of the starting material ascertaining the role of the quarternary ammonium bromide in the reaction. Therefore under the reaction conditions, boron trifluoride etherate being oxophilic could effect concomitant ether cleavage [9,10], rearrangement of the double bond [11] towards the thermodynamically stable product and cyclisation. The plausible mechanism is shown in scheme 2.

Finally, the structure of **2** has also been confirmed by X-ray diffraction analysis. Rod shaped crystals of the isonimbolide were grown from a solution of chloroform: hexane (4:1). It crystallized in $P2_12_12_1$ space group with cell parameters, viz., 9.026 (2), 14.009 (2) and 18.495 (5). The structure was solved using the programme SHELX597 and was refined using SHELXL97 to an *R* factor of 0.065 [12]. The ortep diagram of the molecule is given in figure 1. The bioefficacy of the novel products is being determined. In conclusion, we have developed a simple and mild procedure for obtaining isomers of C-seco limonoids. Such semi-synthetic modifications of natural products gain significance in view of their possible enhanced biological properties.

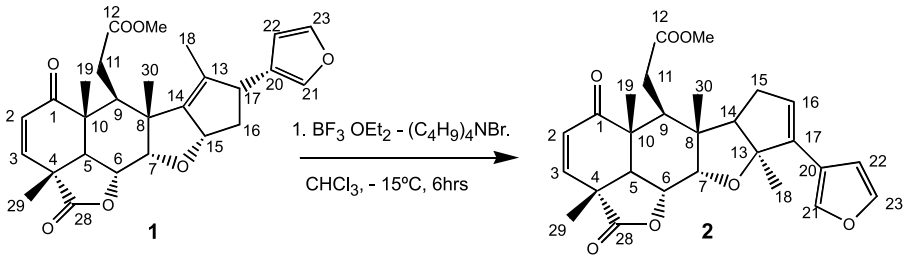
3. Experimental

3.1 General experimental procedures

Melting point was determined using a Raaga industries melting point apparatus and is uncorrected. NMR spectrum was recorded on a Bruker 300 MHz instrument using TMS as

Table 1. The selected ^{13}C NMR data of compounds **1** and **2**.

Carbon	***1***	***2***
7	84.4	77.9
13	136.4	96.0
14	144.6	56.2
15	88.1	31.2
16	40.8	126.2
17	49.0	136.9
18	12.8	24.5

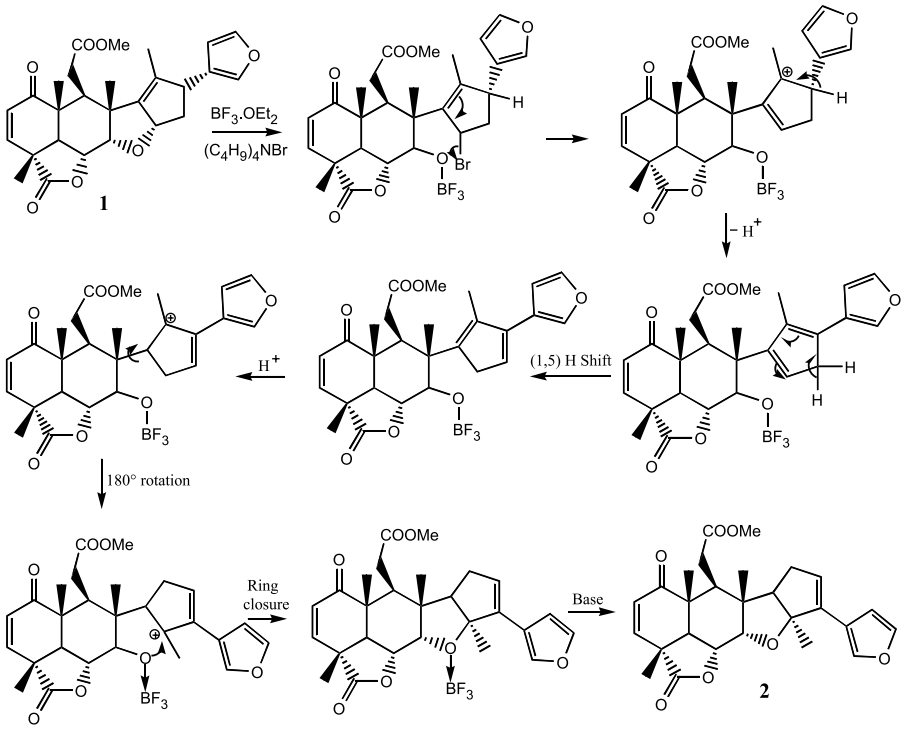


Scheme 1. Conversion of nimbolide (**1**) to isonimbolide (**2**).

an internal standard and $CDCl_3$ as the solvent. Mass spectrum was performed on a Shimadzu QP 1000A and QP 5000 mass spectrometer. High performance liquid chromatography was performed on Shimadzu instrument with LC-10ATVP high pressure pump and a C18 Luna 5μ column (250 × 4.60 mm) and the peaks detected at 215 nm (SPD-10 AVP UV VIS Detector) and the mobile phase being acetonitrile: water system (60:40) at a flow rate of 0.5 ml/min.

3.2 Isolation of nimbolide

Neem leaves were collected, shade dried and extracted with acetone. Column chromatography over 60–120 mesh silica gel using hexane: ethyl acetate eluent yielded pure nimbolide **1** at



Scheme 2. Mechanism of the reaction.

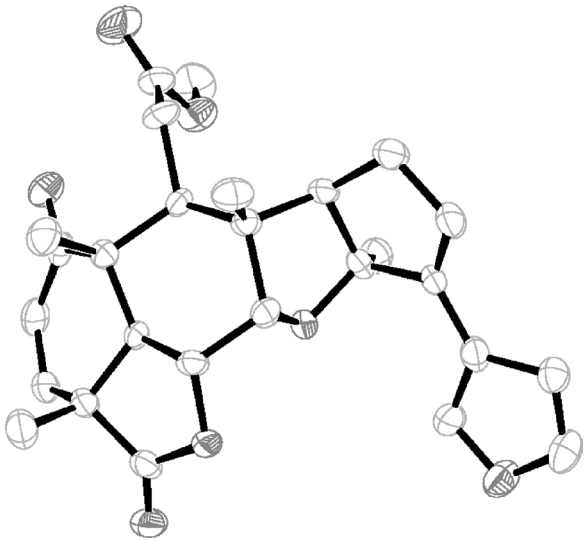


Figure 1. The X-ray structure of compound **2** drawn by ORTEP at 30% probability level (CCDC No. 249735).

a percentage ratio of 1:1. Nimbolide was confirmed by correlating the spectroscopic data with available literature [13].

3.3 Conversion of nimbolide to isonimbolide

1 mmol of nimbolide was dissolved in 450 ml of chloroform (AR) at − 15°C. To this solution 1.05 mmol of tetrabutyl ammonium bromide and boron trifluoride etherate were added. The reaction was allowed to attain room temperature and stirred for 6 hours. The completion of the reaction was monitored by TLC. The reaction was quenched with solid sodium bicarbonate. The reaction mixture was filtered and concentrated under reduced pressure. Flash column chromatography of the crude product over silica gel (70–325mesh) using hexane:ethyl acetate (90:10) furnished the pure product **2** (yield: 52%). A complete assignment of proton and carbon values for isonimbolide is given below.

3.4 Structure determination

$C_{27}H_{30}O_7$ (466); Crystalline, mp 225–235°C, UV (MeOH)λmax: 207 nm.

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.90(1H, d, $J = 9.7$, 2-H); 7.25(1H, d, $J = 9.7$, 3-H); 3.27(1H, d, $J = 12.5$, 5-H); 4.58(1H, dd, $J = 3.8$, 12.5, 6-H); 3.81(1H, s, 7-H); 2.56(1H, dd, $J = 2.6$, 6.6, 9-H); 2.75(1H, dd, $J = 2.6$, 16.5, 11a-H); 2.94(1H, dd, $J = 6.6$, 16.7, 11b-H); 2.40(3H, broad peak, 14-H & 15-H); 5.85(1H, t, $J = 2.7$, 16-H); 1.7(3H, s, 18-H); 1.3(3H, s19-H); 7.3(1H, s, 21-H); 6.5(1H, s, 22-H); 7.8(1H, s, 23-H); 1.44(3H, s, 29-H); 1.34(3H, s, 30-H); 3.77(3H, s, OMe).

^{13}C NMR (300 MHz, $CDCl_3$): δ 200.6(C-1); 130.8(C-2); 149.4(C-3); 43.7(C-4); 47.1(C-5); 73.4(C-6); 77.9(C-7); 52.8(C-8); 44.1(C-9); 46.5(C-10); 31.4(C-11); 173.7(C-12); 96.0(C-13); 56.2(C-14); 31.2(C-15); 126.2(C-16); 136.9(C-17); 24.5(C-18);

15.2(C-19); 118.5(C-20); 140.1(C-21); 109.2(C-22); 142.5(C-23); 175.6(C-28); 18.5(C-29); 17.2(C-30); 51.8(C-OMe).

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