

Photooxygenation of Nimonol, a Tetranortriterpenoid from *Azadirachta indica*. A. Juss.

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Abstract: Nimonol (**1**), a tetranortriterpenoid isolated from the leaves of *Azadirachta indica* A. Juss (Meliaceae), upon photolysis undergoes both Diels-Alder and ene reactions with singlet oxygen at different sites leading to 14,15,20,21-diepoxy-23-nimonolactone (**3**), along with nimonolide (**4**), which have been well-characterised. The novelty of the reported reactions lies in hitherto unreported formation of an α -epoxide in the ring D in tetranortriterpenoids. The photoproduct **4** exhibited antifeedancy comparable to that of azadirachtin-A, the most potent antifeedant constituent isolated from neem.

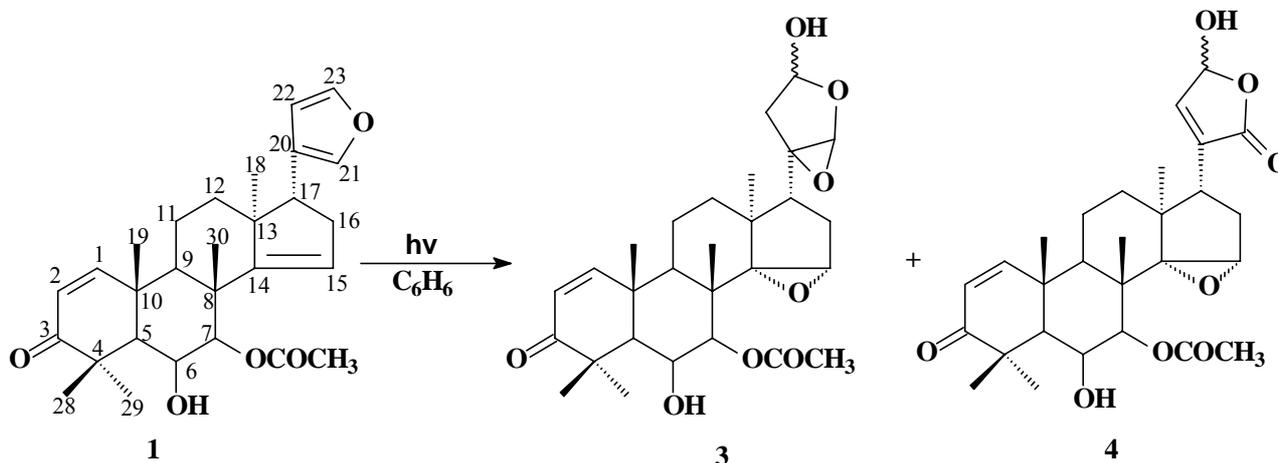
Keywords: Photooxygenation; tetranortriterpenoid; singlet oxygen; antifeedant

Introduction

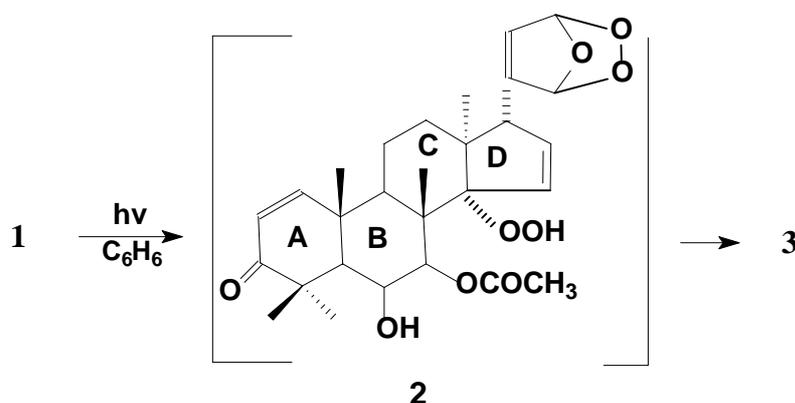
As part of our continuing studies on the photochemistry of triterpenoids of biological importance, herein we report the photooxygenation of nimonol (**1**), a tetranortriterpenoid having both furan and ene systems. Photooxygenation of furans [1] and olefins [2] has drawn considerable attention during the last two decades, as a convenient method for introduction of oxygen at specific sites. A literature survey revealed that reports on the photooxygenation of triterpenoids [3-7] having both furan and olefinic functional groups are scarce.

Results and Discussion

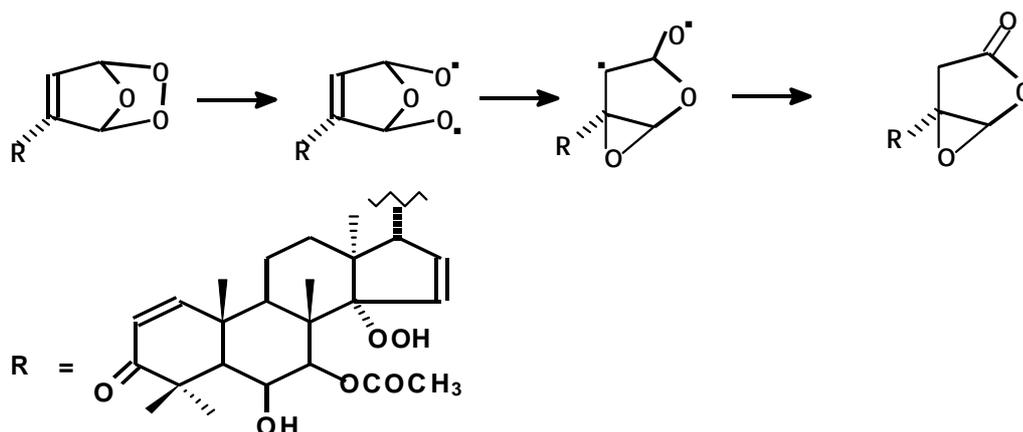
Nimonol (**1**), isolated from uncrushed green leaves of *Azadirachta indica* (Meliaceae) [8] and possessing a β -methyl group at C-13, according to the revised structure [9], was irradiated in benzene in a quartz vessel with a UV lamp (254nm) for six hours until complete decomposition was observed. Purification of the crude product by silica gel column chromatography yielded 14,15,20,21-diepoxy-23-nimonolactone (**3**), followed by nimonolide (**4**).



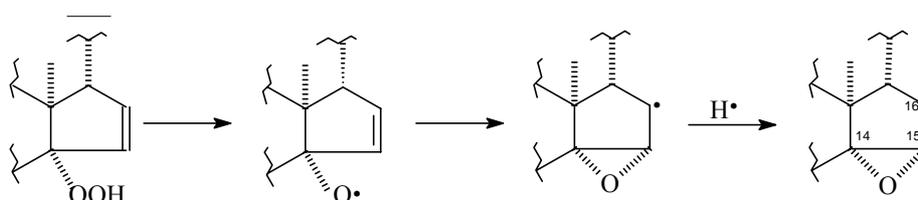
The mechanism for the formation of the photoproduct **3** is envisaged to occur as given below. The formation of the intermediate **2** can be explained by the addition of 1O_2 to furan by a Diels-Alder reaction with 1O_2 acting as the dienophile [1] and the formation of an allylic hydroperoxide at C-14 accompanied by a 1,2-shift of the double bond at C-14 to C-15 in the D ring similar to that of the ene reaction [10-12]. This unstable intermediate then further rearranges to give compound **3**:



In turn, each of the two structural changes can be explained as follows: (a) the possible rearrangement of the endoperoxide functionality to an epoxy lactone in ring E may take place as depicted in the following figure:



and (b), homolysis of the peroxide linkage in the allylic hydroperoxide resulting in the attachment of a C-14 alkoxy radical to C-15, thus forming an epoxide [13] between C-14 and C-15 and at the same time forming a radical at C-16 atom, all followed by abstraction of a hydrogen atom as given below:



A proton elimination from a bridgehead position of an unstable ozonide-like peroxide formed by [4p+2p] cyclo addition of $^1\text{O}_2$ to furan followed by a subsequent rearrangement leads to the formation of photoproduct **4**, nimonolide. The above mechanism has been described in our previous paper [14, 15]. The involvement of singlet oxygen in photolysis reaction and that the singlet oxygen sensitizer is the terpenoid itself has been demonstrated in the case of photooxidation of cedrelone, a tetranortriterpenoid from *Toona Ciliata*. [14].

The spectral data of the photoproduct **3** (^1H and ^{13}C -NMR) was almost identical to that of the starting compound, nimonol (**1**), except the former now possesses an epoxide at the C14–C15 bond in the D ring and an epoxy lactone in the E ring instead of an olefin and furan moieties, respectively. Evidence for these differences came from the following data: (i) one proton [triplet at δ 3.80 ppm ($J = 2.4$ Hz)] attached to carbon at δ 53.7 ppm and attributed to C-15 (^1H - ^{13}C HETERO-COSY) showed ^1H - ^1H COSY connectivities with the methylene protons at δ 2.46 and 2.80 ppm ascribed to the protons at C-16, and a quaternary carbon resonating at δ 77.9 ppm ascertained the presence of an epoxide in the D-ring. The stereochemistry of epoxide is assigned to be α , contrary to the naturally occurring β -epoxy nimonol [16] where the proton at C-15 appears as a singlet, wherein in our case it is triplet; (ii) as for the changes in the furan moiety, the ^{13}C -NMR exhibited signals due to a lactone carbonyl at δ 175.0 ppm (C-23) and a signal at δ 120.4 ppm for a methine carbon (whose proton resonates at δ 5.30 ppm as a singlet) which could be either a dioxygenated or an olefinic methine. The possibility of an olefinic carbon is ruled out as it did not have the counterpart in ^{13}C NMR spectrum, hence the signal

is attributed to a carbon attached to two oxygens (C-21). Further a quaternary carbon at δ 46.9 ppm (C-20) and a methylene at δ 32.9 ppm (the proton of which was observed as a singlet at δ 1.98 ppm, ^1H - ^{13}C HETEROCOSY) explain the presence of a $-\text{CH}_2\text{-CO}$ group. These results all support the presence of an epoxy lactone in the furan moiety.

The ^1H - and ^{13}C -NMR spectrum of compound **4** were similar to those of nimonol (**1**), except for the furan signals. The extra carbonyl resonance at 171.6 ppm indicated an additional lactone carbonyl compared to that of the parent compound. This was confirmed by the presence of IR bands at 1792, 1718 and 1660 cm^{-1} . The absence of C/H signals due to furan moiety indicated that the E-ring (the furan ring) had been the site of attack, similar to the case of photoproduct **3**. The ^{13}C -NMR signal at δ 96.8 ppm (attached to the proton at δ 6.17 ppm), indicated that the carbon must be attached to two oxygen atoms. Further, this proton showed connectivity to the signal at 7.04 ppm (H-22), whose carbon resonates at 146.0 ppm indicating clearly that the carbon connected to two oxygen atoms must be adjacent to an α,β -unsaturated carbon. The presence of two other carbon signals at 137.7 and 171.6 ppm indicated that the furan ring is modified to a δ -hydroxy butenolide moiety. The presence of an additional $-\text{OH}$ was confirmed by the D_2O labile proton at δ 4.91 ppm. The appearance of double signals for these carbons indicated the compound to be an epimeric mixture at C-22. The compound was then assigned to be the photooxygenated nimonol (**1**), named as nimonolide with a molecular formula $\text{C}_{28}\text{H}_{36}\text{O}_7$ (M^+ 484).

Conclusions

Nimonol (**1**), which is a naturally occurring bioactive limonoid with an intact furan ring yields a novel product involving interesting Diels-Alder and ene reactions, on exposure to UV light. The novelty lies in the photolysis of intact limonoids in the absence of a sensitiser and also in the epoxidation of the D-ring under photolysis with an α stereochemistry, which has not been encountered in literature so far. The photoproduct **4**, when tested for antifeedant activity against *Spodoptera litura* (Table 1), exhibited activity comparable to that of Azadirachtin-A while product **3** exhibited activity higher than the parent molecule nimonol [17].

Table 1: Antifeedant activity (%) of limonoids and their photoproducts

S.No	Compounds	5ug	1ug	0.5ug
1	Nimonol	32.2 (2.3)	55.7 (3.2)	32.5(7.6)
2	Photoproduct 4	82.5 (4.2)	80.2 (3.4)	67.5(5.2)
3	Photoproduct 3	73.5 (5.7)	68.7 (7.7)	55.3(4.2)
4	Azadirachtin	100.0 (0)	85.5(2.3)	83.3(1.6)

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Experimental

General

UV spectra (CHCl₃ solutions) were recorded on a Shimadzu 160A instrument. ¹H- and ¹³C-NMR spectra were recorded on a BRUKER 200 MHz instrument at 200 and 50 MHz, respectively, using TMS as the internal standard and CDCl₃ as solvent. Chemical shifts are given in terms of parts per million (δ scale). LRMS were recorded on a Shimadzu QP 5000 mass spectrometer. Precoated silica gel plates (E-Merck, Germany, Art. 5554 Kieselgel 60 F₂₅₄, 0.2 mm thickness) were used for thin layer chromatography. Ammonium molybdate–ceric sulphate in H₂SO₄ was used as the developing agent. Analytical HPLC was carried out using a Shimadzu LC 8A system linked to a CR4A data processor. Peaks were detected at 215 nm. A Shimpack reverse phase (C18, 25 cm x 4.6 mm i.d.) analytical column and 60:40 methanol-water as eluent were used for the analyses. Silica gel (70-325 mesh) was used for flash column chromatography with 2:3 hexane-ethyl acetate as eluent. Photochemical reactions were carried out in a HEAOP compact photolysis apparatus model 141 supplied by HEBER Scientific, Chennai (India) and containing 8 x 8 W lamps (UV 254 nm).

Irradiation of Nimonol

An air saturated solution of Nimonol (**1**) (100 mg, λ_{\max} 288, 244 nm) in benzene (200 mL) was irradiated with a UV lamp system (254nm, 8 x 8 W) for six hours at the end of which the complete decomposition of nimonol **1** was confirmed by TLC (eluent: 60% ethyl acetate in *n*-hexane). The solvent was removed under reduced pressure. The product was chromatographed on silica gel (flash, 70-325 mesh; 40% ethyl acetate in hexane) under nitrogen atmosphere to yield compound **3** (40 mg, 41%) followed by compound **4** (24 mg, 28.6 %).

Analytical and Spectral Data.

Compound 3: Mp 124°C; UV λ_{\max} (nm): 251 (ϵ =7651) and 254 (ϵ =7021); IR ν_{\max} / CHCl₃/cm⁻¹: 816, 1752, 1718, 1660, 1593, 1449, 1372, 1254, 1084, 1062, 646; ¹H-NMR (δ): 7.05 (1H, *d*, J 10, H-1), 5.84 (1H, *d*, J 10, H-2), 5.33 (1H, *d*, J 5.3, H-7), 5.30 (1H, *s*, H-21), 4.30 (1H, *dd*, J 11.4, 3, H-6), 3.80 (1H, *t*, J 2.4, H-15), 2.86 (1H, *d*, J 3.5, H-17), 2.80 (1H, *m*, H-16a), 2.46 (1H, *m*, H-16b), 2.27 (1H, *m*, H-9), 2.23 (1H, *d*, J 4.9, H-5), 2.05 (1H, *m*, H-11a), 1.98 (2H, *s*, H-22), 1.98 (3H, *s*, -OCOCH₃), 1.96 (2H, *m*, H-12), 1.67 (1H, *m*, H-11b), 1.33 (3H, *s*, Me), 1.24 (3H, *s*, Me), 1.17 (3H, *s*, Me), 1.13

(3H, s, Me), 1.05 (3H, s, Me); $^{13}\text{C-NMR}$ (δ): 205.0 (C-3), 171.8 (-OCOCH₃), 157.4 (C-1), 126.1(C-2), 120.4 (C-21), 175.0 (C-23), 78.9 (C-7), 77.9 (C-14), 67.9 (C-6), 53.7 (C-15), 51.0 (C-13), 49.8 (C-5), 46.9 (C-20), 45.4 (C-8), 43.9 (C-10), 43.0 (C-17), 40.4 (C-4), 37.1(C-9), 33.7 (C-16), 32.9 (C-22), 31.8 (Me), 29.6 (C-12), 27.8 (Me), 21.1 (-OCOCH₃), 20.8 (Me), 20.1(Me), 20.1(Me), 16.4 (C-11); EI/MS m/z 500 (M^+).

Compound 4: Mp 136-138°C; UV λ_{max} (nm): 240 ($\epsilon=9448$), and 254 ($\epsilon=8109$); IR ν_{max} (cm^{-1}): 3352, 2975, 2860, 1792, 1752, 1718, 1698, 1583, 1445, 1310, 1024, 970, 880, 842, 647, 611; $^1\text{H-NMR}$ δ : 7.12 (1H, *d*, J 10, H-1), 7.04 (1H, *d*, J 7, H-21), 6.17 (*d*,1H, J 7, H-22), 5.89 (1H, *d*, J 10, H-2), 5.37 (1H, *t*, J 7, H-15), 5.37 (1H, *d*, J 2.8, H-7), 4.91 (*brs*, D₂O exchangeable, 1H, -OH), 4.37 (1H, *dd*, J 11.6, 3, H-6), 2.81 (1H, *m*, H-17), 2.57 (1H, *m*, H-12a), 2.44 (1H, *d*, J 11.6, H-9), 2.23 (1H, *m*, H-5), 2.17 (1H, *m*, H-12b), 2.05 (*s*, 3H, -O-COCH₃), 2.04 (1H, *m*, H-16a), 1.75–1.65 (1H, *brs*, D₂O exchangeable, -OH), 1.76 (1H, *m*, H-16b), 1.81 - 1.72 (2H, *m*, H-11), 1.40 (3H, *s*, Me), 1.30 (3H, *s*, Me), 1.27 (3H, *s*, Me), 1.13 (3H, *s*, Me), 0.90 (*s*,3H, Me); $^{13}\text{C-NMR}$ δ : 206.2 (C-3), 172.0 (-OCOCH₃), 171.6, 171.4 (C-21), 158.5 (C-14), 157.6 (C-1), 146.0, 145.7 (C-22), 137.7, 137.6 (C-20), 126.1(C-2), 119.3 (C-15), 96.8, 96.4 (C-23), 79.1(C-7), 68.1(C-6), 50.6 (C-17), 49.9 (C-5), 47.2 (C-13), 45.5 (C-8), 43.3 (C-10), 40.6 (C-4), 36.9 (C-9), 34.3 (C-16), 32.7 (C-12), 31.9 (Me), 27.3 (Me), 21.2 (-OCOCH₃), 21.1 (Me), 20.9 (Me), 20.3 (Me), 16.4 (C-11); EI/MS m/z 484 (M^+).

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Sample Availability: Samples of compounds **1** (20 mg), **3** and **4** (3 mg.) are available from the authors.

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