

Photomediated Transformation of Salannin, a Tetranortriterpenoid from *Azadirachta indica* A. Juss

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Abstract: Photolysis of Salannin (**1**), an important bioactive compound from *Azadirachta indica* A.Juss (Neem), affords Δ^{17} -isosalanninolide (**2**), a hitherto unknown compound, along with isosalanninolide (**3**) and salanninolide (**4**). A probable mechanism has been suggested. While the mechanism of formation of **2** involves both a [4+2] cycloaddition and a [1,3] sigmatropic shift in the furan and D-rings, respectively, formation of **3** and **4** involves the decomposition of an ozonide-like peroxide intermediate.

Keywords: *Azadirachta indica*, Salannin, cycloaddition, sigmatropic shift, antifeedant

Introduction

Neem, *Azadirachta indica* A. Juss (Meliaceae) seeds contain a number of complex triterpenoids which are of great interest as they exhibit a variety of biological properties [1-4]. Several of these contain UV chromophores such as α,β - unsaturated ketone, furan, α,β -unsaturated ester and tigloyl

groups. The bio-efficacy of neem formulations is altered due to degradation of the triterpenoids under light exposure [5,6]. Hence, it is relevant to study the formation, isolation and bio-activity of the products formed when pure samples of these limonoids are exposed to sunlight. Herein the photolysis of salannin, one of the major triterpenoids present in the neem seed is reported.

Irradiation of salannin (**1**) using a sunlamp with a wider spectrum of irradiation equivalent to exposure to sunlight yielded three compounds (Figure 1). The present paper describes the sequence of events leading to the formation of Δ^{17} -isosalanninolide (**2**) and its complete characterization. It may be noted that the compound **2** exhibited antifeedancy against *Spodoptera litura* comparable to Azadirachtin A, the most potent antifeedant in neem extract. Compounds isosalanninolide (**3**) and salanninolide (**4**) [7] have been previously reported to be formed in the photolysis of salannin (**1**) using UV irradiation in presence of oxygen.

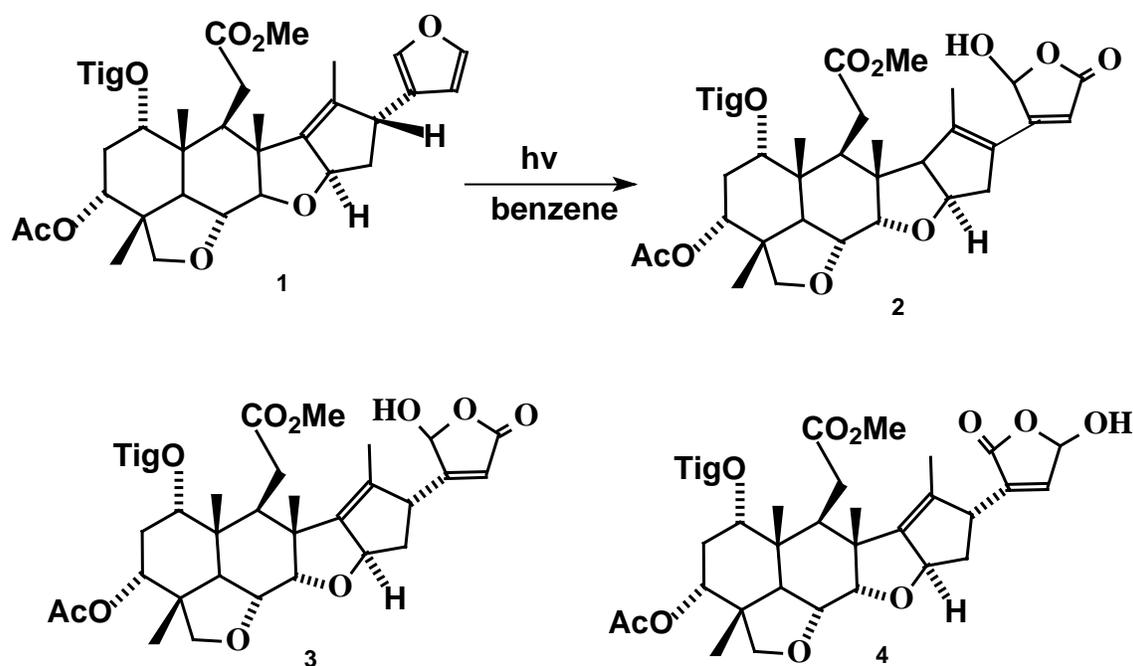


Figure 1

Results and Discussion

Salannin (**1**) isolated from seed kernel of *A. indica* [8,9] was irradiated using a sunlamp in benzene for eight hours. Purification of the crude product by HPLC yielded compounds **2**, **3** and **4**. The mechanism for the formation of **2** is envisaged as given below (Figure 2) while the mechanism for **3** and **4** is assumed to be similar to that of the reported photooxygenation of cedrelone[10].

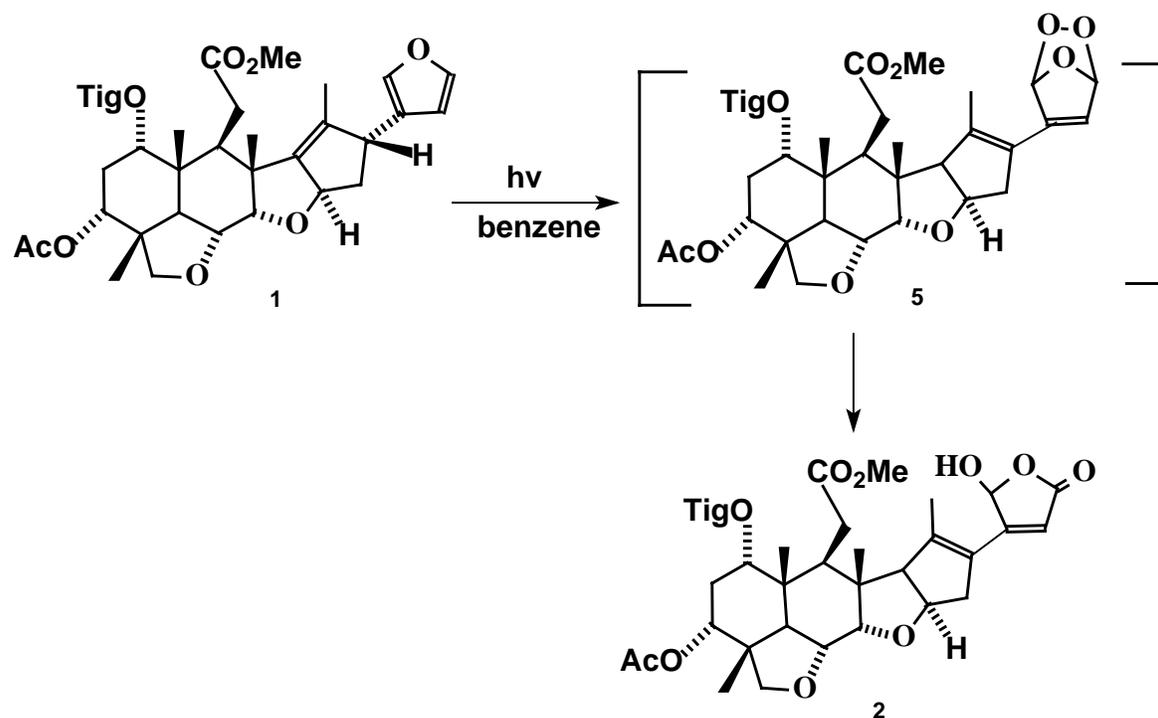


Figure 2

The formation of the intermediate **5** can be explained by: a) the addition of $^1\text{O}_2$ to furan generating an unstable endoperoxide similar to that of Diels-Alder reaction.[11]; b) [1,3] suprafacial sigmatropic hydrogen shift resulting in the 1,2-shift of the double bond in the D-ring. Further the unstable endoperoxide functionality in compound **5** rearranges to yield hydroxy butenolide in ring E of compound **2** [12].

The structure of the compound **2** was established on the basis of the following spectral evidence. Compound **2** exhibited a λ_{max} at 298, 227 nm ($\log \epsilon$ 3.782, 4.395) indicating an extra conjugation, compared to salannin (**1**) (λ_{max} at 225 nm, $\log \epsilon$ 4.356). The infrared spectrum of **2** showed additional absorptions at 1708 cm^{-1} (α,β - unsaturated ketone) and 3376 cm^{-1} (hydroxyl group). The ^1H - and ^{13}C -NMR spectrum of **2** was similar to that of **1** except for changes observed in the D and furan rings. The ^{13}C -NMR exhibited signals due to a lactone (169.2 ppm), a deoxygenated methine at 88.1 ppm (proton of which resonated at 5.52 ppm as a triplet), an allylic methylene at 31.1 ppm (attached to protons at 2.31 and 2.37 ppm as multiplets) and 4 olefinic carbons (154.0, 119.5, 130.9, 98.1) among which only the signal at 98.1 showed connectivity with a hydrogen at 5.92 ppm (as a singlet). The changes observed in the olefinic region indicated that the double bond between C-13 and C-14 has now been reorganized to C-13 and C-17 and is in conjugation with C-20, C-22 which lie in the same plane. The

D₂O exchanged ¹H-NMR spectrum revealed the presence of a -OH at 5.93 ppm, The structure assigned was further supported by the mass spectra of **2** which showed a M⁺ at m/z 628.

It must be noted that though salannin **1**, the major constituent in neem formulations, did not exhibit significant antifeedant activity against *Spodoptera litura*, its photoproduct **2** exhibits remarkable activity comparable to that of Azadirachtin A, the results of which are published elsewhere[13].

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Experimental

General

UV spectra were recorded on a Shimadzu 160A instrument. NMR spectra were recorded on a BRUKER 200 MHz instrument 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 visualization agent.

Analytical high performance liquid chromatography was carried out using a C₁₈ RP column (E-Merck, 10 μ m, 4.6 mm x 25 cm) eluting with CH₃CN: H₂O (50: 50) at a flow rate of 1 mL/min and UV detection (215 nm). For semi-preparative HPLC runs, a C₁₈ RP column (E-Merck, 10 μ m, 9.6 mm x 25 cm) was used with CH₃CN: H₂O (50: 50) as eluant at a flow rate of 5 mL/min and the peaks were again detected at 215 nm.

Irradiation of Salannin (**1**).

Salannin (**1**) (50mg) dissolved in benzene (80mL) was irradiated with a sunlamp equipped with a cooling device (Phillips, 300W) under aerated conditions for eight hours, at the end of which complete decomposition of salannin was noted by analytical HPLC. The solvent was removed under reduced pressure and the products were purified by semi-preparative HPLC. Three major peaks eluted with retention times of 5.6, 7.4 and 15.7 min., respectively. The fractions with R_t 5.6 and 15.1min. were

identified as compounds **3** (8mg) and **4** (14mg) by comparison with reported data [7]. The fraction with R_t 7.4 min yielded **2** as a white solid (12 mg) upon concentration.

Analytical and spectral data of compound **2**.

Mp 114 - 116°C; UV: λ_{\max} (MeOH) / nm 298 (log ϵ 3.782), 227 (log ϵ 4.395); IR: ν_{\max} / cm^{-1} 3376, 2976, 2880, 1760, 1708, 1696, 1651, 1596, 1488, 1465, 1379, 1315, 1273, 1068, 1001, 969, 880, 838, 646, 611; $^1\text{H-NMR}$ (200MHz: CDCl_3 ; Me_4Si): δ_{H} 6.93 (1H, m, 3'-H), 5.93 (1H, br s, -OH), 5.92 (1H, s, 22-H), 5.52 (1H, t, 21-H), 4.95 (1H, t, 1-H), 4.71 (1H, t, 3-H), 4.24 (1H, d, J 3.0, 7-H), 3.98 (1H, dd, J 9.4 and 3.1, 6-H), 3.79 (1H, d, J 8.9, 15-H), 3.66 (1H, d, J 7.5, 28b-H), 3.58 (1H, d, J 7.5, 28a-H), 3.52 (3H, s, COOMe), 2.68 (1H, d, J 9.1, 5-H), 2.66 (2H, m, 9-H and 14-H), 2.37 (2H, m, 11b-H and 16b-H), 2.31 (2H, m, 11a-H and 16a-H), 2.21 (2H, m, 2-H), 1.93 (3H, s, OCOMe), 1.87 (3H, s, Me), 1.81 (3H, s, Me), 1.73 (3H, s, Me), 1.28 (3H, s, Me), 1.23 (3H, s, Me), 1.03 (3H, s, Me); $^{13}\text{C-NMR}$ (50MHz: CDCl_3 ; Me_4Si): δ_{C} 174.19 (C-12), 170.33 (OCOMe), 169.20 (C-23), 166.54 (C-1'), 154.00 (C-13), 137.99 (C-3'), 130.85 (C-20), 128.74 (C-2'), 119.53 (C-17), 98.12 (C-22), 88.09 (C-21), 85.82 (C-7), 77.63 (C-28), 72.39 (C-6), 71.48 (C-3), 71.21 (C-1), 52.47 (C-15 and COOMe), 49.56 (C-8), 42.67 (C-4), 40.57 (C-10), 40.05 (C-5), 39.51 (C-9 and C-14), 31.13 (C-11 and C-16), 27.28 (C-2), 20.82 (OCOMe), 19.65 (C-29), 16.79 (C-30), 14.87 (C-19), 14.43 (C-4'), 13.03 (C-18), 11.86 (C-5'). Mass spectrum: m/z 628 (M^+).

References

1. Kraus, W., in *The Neem Tree, Azadirachta indica A.Juss and other Meliaceous Plants*, H. Schmutterer, ed, Verlag Chemie: Weinheim, **1995**; pp. 35-38.
2. Blaney, W.M.; Simmonds, M.S.J.; Ley, S.V.; Anderson, J.C.; Toogood, P.L. Antifeedant effects of Azadirachtin and structurally related compounds on lepidoptera larvae, *Entomol. Exp. Appl.* **1990**, *55*, 149-160
3. Butterworth, J.H.; Morgan, E.D. Isolation of a substance that suppresses feeding in locust, *J. Chem. Soc. Chem. Commun.*, **1968**, 23-24.
4. Govindachari, T.R.; Narasimhan N.S.; Suresh G.; Partho, P.D.; Geetha Gopalakrishnan, Insect antifeedant and growth regulating activities of salannin and other C-seco limonoids from neem oil in relation to azadirachtin *J. Chem. Ecol.*, **1996**, *22*, 1453-1461.
5. Barnaby, M.A.; Yamasaki, R.B.; Klocke, J.A. Biological activity of azadirachtin, three derivatives, and their ultraviolet radiation degradation products against tobacco bud worm (Lepidoptera: Noctuidae) larvae. *J. Econ. Entomol.*, **1989**, *82*, 58-63.

6. Stokes, J.B.; Redfern, R.E. Effect of sunlight on azadirachtin: antifeeding potency. *J. Environ. Sci. Health*, **1982**, *A17*, 57-65
7. Jarvis, A. P. ; Johnson,S. ; Morgan, E.D.; Simmonds, M.S.J.; Blaney, W.M., Photooxidation of nimbin and salannin-tetranortriterpenoid from neem tree, *J. Chem. Ecol.*, **1997**, *23*, 2841-2860.
8. Henderson,R.; McCrindle, R.; Melera,A.;Overton, K.W., Tetranortriterpenoids-IX The constitution and stereochemistry of salannin, *Tetrahedron*, **1968**, *24*, 1525-1528
9. Govindachari, T.R.; Suresh, G.; Geetha Gopalakrishnan, A direct preparative high performance liquid chromatography procedure for the isolation of major triterpenoids and their quantitative determination in neem oil. *J. Liq. Chromatogra.*, **1995**, *18*, 3465-3471
10. Geetha Goplakrishnan.; Pradeep Singh, N.D.; Kasinath, V.; Malathi, R.; Rajan,S.S. Photooxidation of cedrelone,a tetranortriterpenoid from *Toona ciliata*, *Photochem. Photobio* **2000**, *72*, 464-466
11. Foote,C.S.; Wuesthoff, M.T.; Wexler,S.;Burstain,I.G.; Denny, R.; Schenck, G.O.;Schulte-Elte, K-M. Photosensitized oxigenation of alkyl substituted furans, *Tetrahedron* **1967**, *23*, 2583-2599.
12. Gilbert,A., in *Photochemistry in Organic Synthesis*, Coyle, J.D., ed.; Royal Society of Chemistry:Cambridge, U.K. **1986**; pp. 141-163
13. Pradeep Singh , N.D.; Ph.D. Thesis, University of Madras, November **2000**.

Sample Availability: Both the starting compound (50 mg.) and the photoproducts (5 mg. each) are available from the author.