Ratio of products formed on photo-oxidation of the neem triterpenoids nimbin and salannin

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Dedicated to Gurnos Jones on his seventieth birthday

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Abstract

Two isomeric products containing a hydroxybutenolide, formed from the furan ring, are produced by the photo-oxidation of the neem triterpenoid nimbin, an analogous pair of isomers are similarly formed by oxidation of salannin. The relative amounts of these products can be varied by the addition of a hindered base either during or after the oxidation. Addition of DABCO after the oxidation can increase appreciably the proportion of the minor isomer, the biologically more valuable products, from both reactions.

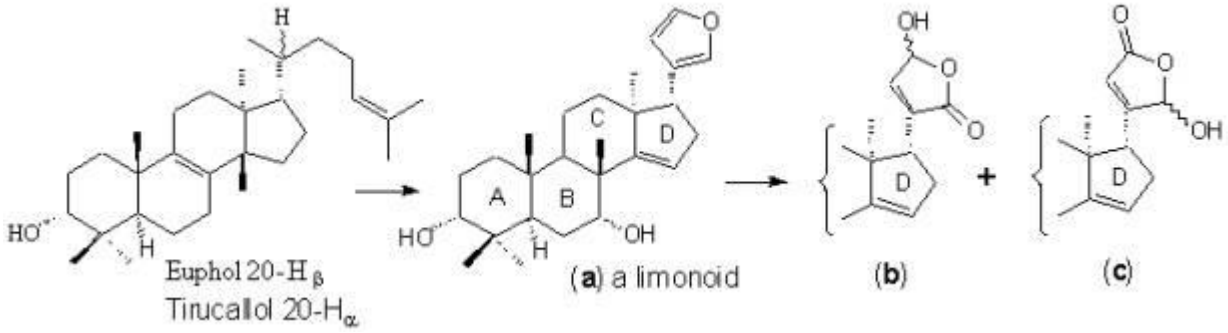
Keywords: Furans, nimbin, DABCO, triterpenoids

Introduction

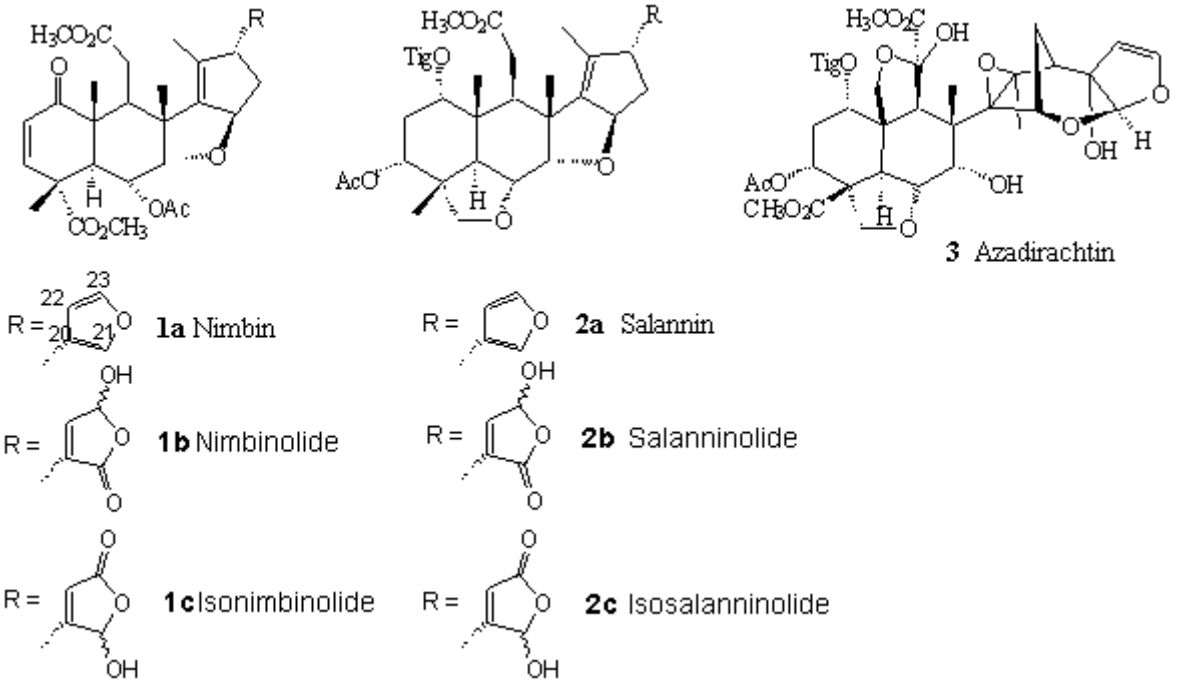
The great interest in the substance azadirachtin, the powerful natural pesticide from the seeds and other parts of the neem tree (*Azadirachta indica* A. Juss.),[1] and related compounds, has stimulated the investigation of the triterpenoids of this plant. At a recent count, at least 145 terpenoid substances had been identified in various parts of this one species. A large number of the compounds belong to the class of limonoids, in which the side chain has been shortened and cyclized to a furan ring (a) (Scheme 1).[2] A further, smaller group, containing a butenolide ring (b) or (c) in place of the furan appear to be oxidation products of the latter. The structures of the known compounds were reviewed to 1994.[3] There has been debate as to whether these oxidation products arise during isolation of the parent substances or are formed in the plant. The argument

has been put forward that the ratio of products (b) and (c) in scheme 1 are different, when obtained from the plant or produced chemically from the parent furan (a).[4,5]

Compound 2b was first isolated from the seeds of *Melia dubia* and called compositolide,[6] and was also produced by oxidizing salannin by light and oxygen.[7] It was also isolated from *Azadirachta indica* and called salannolide.[8] Isosalanninolide was first isolated in nature by Jarvis[7] from neem seeds. The nimbin product 1b was called isophotonimbin when isolated from neem seeds,[9] and the isomer 1cwas first found in the stem bark of the neem tree.[10]



Scheme 1



Scheme 3

As part of our continuing structure-activity-relationship studies, we have examined the photo-oxidation products of two of the most abundant triterpenoids in neem seeds, nimbin 1a and

salannin 2a, and shown that the isomeric products nimbolide 1b and isonimbolide 1c, and salanninolide 2b and isosalanninolide 2c, have interesting properties affecting feeding and development of insects.[11] Both isonimbinolide 1c and isosalanninolide 2c are potent antifeedants towards the armyworm *Spodoptera littoralis* and the desert locust *Schistocerca gregaria*, and isonimbinolide has been found to be as efficient at disrupting the growth of *S. littoralis* as the most potent natural compound azadirachtin 3.[7-11] These compounds were more active than their 23-hydroxy-21-butenolide isomers, but it is the latter, less active isomers that are formed in greater proportion under the photo-oxidation conditions in our earlier work.[11] The ratio of products (b) and (c) obtained does not indicate whether they are formed inside the plant or during chemical isolation.

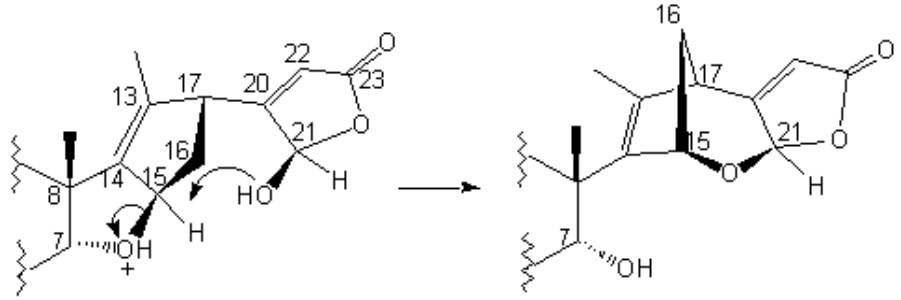
It is tempting to speculate about the reason for the high potency of isonimbinolide and isosalanninolide. We believe that it is due to them having the correct geometry to undergo an acid catalysed rearrangement *in vivo* to produce a bicyclic ring system almost identical to that of azadirachtin (Scheme 2). Protonation of the bridging oxygen between C-15 and C-7 will result in a partial carbocation at C-15, which is stabilized by the adjacent double bond and is susceptible to nucleophilic attack. Molecular models show that either epimer at C-21 (which is also epimerizable) is ideally placed to attack C-15 in an S_N2 manner, inverting configuration and thus forming the bicycle. This also reveals the C-7 hydroxyl which in azadirachtin is known to be crucial for high activity. We hope to prove this mechanism by a study of biomimetic rearrangements of these compounds using Lewis acid catalysts.

The desire to investigate these compounds further led us to undertake studies into their preparation. We hoped that by altering conditions it is possible to alter the proportions of the normal and iso-products in the photo-oxidation of both nimbin and salannin.

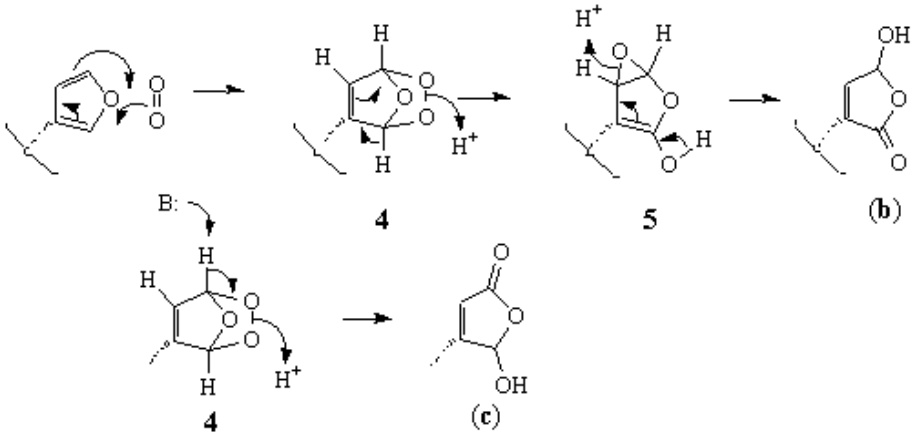
Results and Discussion

In the conditions used in our earlier work[11] the biologically less active 23-hydroxy-21-butenolide isomers are formed in greater proportion under the photo-oxidation conditions. The preference for the formation of these 23-hydroxy-21-butenolide isomers can be explained from the proposed mechanism for the photolysis of the furan rings. It is believed that such a reaction occurs *via* a [4 + 2] cycloaddition of singlet oxygen to the ring, initially giving an ozonide-type intermediate (Scheme 3). Attack can occur from above or below the plane of the furan ring. In their study of the marine natural product ambliol A, Kernan and Faulkner[12] showed that the ozonide intermediate, formed in the photolysis of the furan ring, rearranged to give two cis-epoxides (at C-22,23 or C-20,21) and four epoxylactones. These epoxylactones further rearranged on standing, treatment with acid, or chromatography on silica gel to give the two hydroxybutenolides. In a study of simpler furans, it has been proposed that the thermal rearrangement of the ozonide occurs via a transition state, the products of which are the enol forms of the epoxylactones (e.g. 5), which are the immediate precursors of the

hydroxybutenolides.[13] The presence of the bulky A-to-D-ring system at C-17 of the ozonide intermediates (4) of nimbin and salannin may cause steric hindrance towards formation of the C-O σ-bond of the transition state,[12] required to give the epoxy-lactone precursor of the 21-hydroxy-23-butenolides.



Scheme 2



Scheme 3

On the other hand, the formation of these isomers requires the regiospecific removal of the hydrogen at C-23 of the ozonide.[12] We reasoned that this could be achieved by treatment with a hindered base and we have carried out experiments with DABCO and triethylamine added at the start or at the end of the oxidation. The results of these experiments are detailed in Tables 1 and 2.

Table 1. Amounts and ratios of nimbinolide and isonimbinolide, formed from the photolysis of nimbin under various conditions

Conditions used	Reaction time (min)	Amount of nimbinolide (mg)	Amount of isonimbinolide (mg)	Total yield (%)	Ratio nimbinolide : isonimbinolide
No base added	75	2.88	1.48	12	66:34
NEt_3 added at start	135	1.74	3.62	14	32:68

DABCO added at start	180	1.71	4.21	16	29:71
DABCO added at end	75+15	3.40	2.98	15	53:47

Table 2. Amounts and ratios of salanninolide and isosalanninolide, formed from the photolysis of salannin under various conditions

Conditions used	Reaction time (min)	Amount of salanninolide (mg)	Amount of isosalanninolide (mg)	Total yield(%)	Ratio salanninolide : isosalanninolide
No base added	45	4.63	0.93	14	84:16
Silica added at end	45+15	4.12	0.69	12	86:14
NEt_3 added at start	60	3.92	1.30	13	75:25
DABCO added at start	75	2.30	1.44	9	62:38
DABCO added at end	45+15	3.93	1.12	12	72:28

The photo-degradation of nimbin 1a was followed by analysis of the mixture at 15 min intervals using supercritical fluid chromatography (SFC). As the peak for nimbin, which eluted at about 2.9 min (Figure 1a), decreased, a number of new compounds eluting between 4.0 min and 8.0 min were detected (Figure 1 b-f). Once all the nimbin had disappeared, (after 75 min), 1,4-diazabicyclo[2,2,2]octane (DABCO) was added to the reaction mixture. After stirring for 15 min, SFC analysis of the concentrated mixture showed the appearance of two main UV-absorbing compounds at retention times of 9.6 min and 14.3 min (Figure 1g). These were isolated and identified by their ^{1}H NMR and IR spectra and chromatographic retention times as nimbinolide 1b and isonimbinolide 1c.

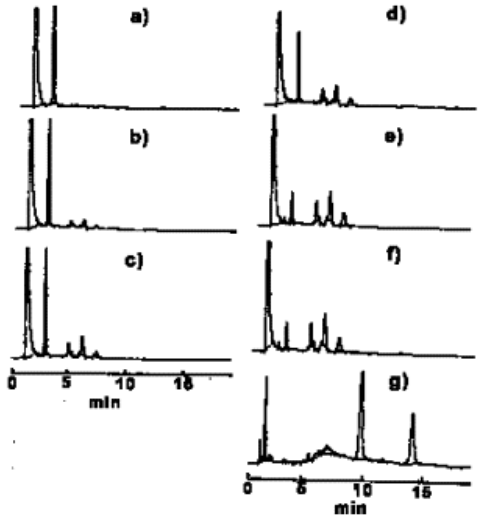


Figure 1. Supercritical fluid chromatograms of the course of photo-oxidation of nimbin, at a) zero time, b) 15 min, c) 30 min, d) 45 min, e) 60 min, f) 75 min and g) after the addition of

DABCO and chromatography on a silica gel Chromatotron plate. The two large peaks in g) are from the left, nimbinolide and isonimbinolide.

Salannin 2a was subjected to conditions similar to nimbin, with the degradation of salannin in the reaction mixture being monitored by SFC at 15 min intervals, for 45 min. The photolysis of salannin (t_R ~ 3.5 min) also led to the formation of more polar compounds, which eluted between 5.0 min and 11.0 min by SFC. Stirring this mixture with DABCO caused the formation of two new UV-absorbing compounds, which had retention times of 12.2 min and 13.9 min. These were isolated by chromtography on a silica Chromatotron plate, and identified by their ^{1}H NMR and IR spectra and retention times as salanninolide 2b and isosalanninolide 2c respectively.

The addition of a hindered base to the solution of nimbin or salannin at the start of the photolysis, caused an increase in the amount of the 21-hydroxy-23-butenolide isomers, compared to when the reaction was carried out in the absence of base. With the added base, however, the rate of photo-degradation of the starting materials was greatly reduced (see tables 1 and 2), since the bases used (DABCO and triethylamine) also act as singlet oxygen quenchers. Introducing the base to the reaction mixture after all the starting material had been consumed gave a faster reaction rate. Addition of the base at this point yielded more of the isonimbinolide and isosalanninolide compared to when no base was used, but the yields were less than when the base was added at the start of the reaction. Likewise, the ratio of these two compounds over their isomers (nimbinolide and salanninolide, respectively) was greater compared to that when no base was added, but less than when the base was added to the mixture at the start of the reaction. It is thought that DABCO acts as a bulky base and extracts the less hindered proton from C-23 of the ozonide intermediate 4, which would explain the increase in the formation of the 21-hydroxy-23-butenolide isomers under such conditions. These results suggest that the ozonide is only partially transformed to the epoxylactones during the photolysis in the absence of base. A portion of the ozonide must still be present at the end of the photolysis, which can then react with the base. In all conditions it is more difficult to form the iso-product of salannin than it is for nimbin.

Further attempts were made to improve the yields of the reactions by changing the solvent. Dichloromethane and THF gave no identifiable products, whereas toluene as solvent gave no reaction. Rose bengal was used as a UV-sensitizer, however again large product mixtures were obtained, even at low temperatures (-40 ^{o}C), and no reaction was observed when a less intense light source (200 W tungsten lamp) was used. After these attempts the work was halted.

Another group has reported the photo-oxidation of nimbin in chloroform and salannin in methanol with rose bengal added as a UV sensitizer and a more powerful 200 W Hanovia lamp.[14] Oxidation of nimbin required 10 h and gave a total yield of 73% of the two compounds 1b and 1c and a ratio isomers of 53:47. It is difficult to compare this with our work since the products were obtained as a viscous oil and a gum. Oxidation of salannin for 1 h gave a yield of 56% of 2b and 2c combined and a ratio of products of 42:58. In all conditions it is more difficult to form the iso-product of salannin than it is for nimbin.

Experimental Section

Supercritical fluid chromatography. The supercritical fluid chromatograph consisted of two constant pressure delivery systems (Milton Roy, Stone, UK), one a methanol pump (ConstaMetric 3000), and the other a liquid carbon dioxide pump (CP 3000). Whilst being pumped, the carbon dioxide was kept in a liquid state, using a chiller (Haake, Karlsruhe, Germany) set at -10 ^{o}C. The mobile phase flow was controlled by a gradient programmer (GM 4000, Milton Roy). The methanol and carbon dioxide were mixed using a high pressure mixer (Oriental Motor Co., Japan), and passed into an oven heated to 55 ^{o}C, to give the supercritical fluid, the pressure of which was controlled to approximately 3000 psi with a back pressure regulator (Tescom, Elk River, USA). Samples were injected through a 20 mL loop (Rheodyne, Cotati, USA) connected to a pre-column (Upchurch Scientific, Oak Habour, USA), and the compounds were analysed with a Spherisorb cyanopropyl silica column (Phase Separations, Deeside, UK), of particle size 5 mm (150 x 4.6 mm i.d.). Separations were performed under isocratic conditions at a flow rate of 2 mL/min, with a mobile phase of carbon dioxide-methanol (94:6 v/v). Samples were detected using a variable wavelength UV detector (Milton Roy), set at 217 nm and data collection was done using an R-3A computing integrator (Shimadzu, Kyoto, Japan).

Photo-oxidation. Nimbin and salannin were extracted from neem seeds of Indian origin, as described by the procedure of Johnson and Morgan.[15] Photo-oxidation of nimbin and salannin were carried out using the method described by Jarvis *et al*.[11] Nimbin (36 mg, 0.067 mmol) was dissolved in benzene (45 ml), in a Schenk photo-chemical reactor. Oxygen was bubbled through the solution whilst it was being irradiated with UV light (Hanovia 1L, 90 W, Englehard, Slough, UK). The reaction was followed by supercritical fluid chromatography (SFC), and stopped once all the nimbin had reacted. DABCO (40 mg) was added, and the mixture stirred for 15 min. The benzene was removed by rotary evaporation, the residue re-dissolved in dichloromethane (1 mL), and chromatographed on a 2 mm thick silica gel (2 g) (Merck, Darmstadt, Germany) Chromatotron plate (Harrison Research, California, USA). The plate was eluted with chloroform (100 ml), methanol-chloroform (5:95, 100 mL) and methanol (100 mL). The methanol-chloroform fraction contained two main UV-absorbing compounds by SFC, which were separated by chromatography across a second silica gel (1 mm, 50 g) Chromatotron plate, the compounds being eluted with methanol-chloroform (3:97). The faster running compound was identified as nimbinolide (3.40 mg) and the slower running compound as isonimbinolide (2.98 mg). The ^{1}H NMR and IR spectroscopic data of the two compounds were identical to that given in the literature.[11]

The irradiation experiment was then repeated using salannin (40 mg, 0.067 mmol) in benzene. Once all the salannin had disappeared, DABCO (40 mg) was added and the products isolated as above. The Chromatotron plate was eluted with chloroform (100 mL) and then methanol

(100 mL). The methanol fraction contained two main UV-absorbing compounds by SFC, which were separated on a second silica gel Chromatotron plate. The compounds were eluted with methanol-chloroform (3:97). The faster running compound was identified as salanninolide (3.93 mg) and the slower running compound as isosalanninolide (1.12 mg). The ^{1}H NMR and IR spectroscopic data was identical to that given in the literature.[11]

Acknowledgements

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References

1. Schmutterer, H., Ed.; *The Neem Tree;* VCH: Weinheim and New York, 1995; p 696.
2. Buchanan, J.G.St.C.; Halsall, T.G. *J. Chem. Soc. (C)* **1970**, 2280.
3. Kraus, W. In *The Neem Tree;* Schmutterer, H. Ed.; VCH: Weinheim and New York, 1995; p 35.
4. Grimminger, W.; Kraus, W. *Liebigs Ann. Chem.* **1979**, 1571.
5. Siddiqui, S.; Siddiqui, B. S.; Faizi, S.; Mahmood, T. *J. Nat. Prod.* **1988**, *51*, 30, and other papers from this group listed therein.
6. Purushothaman, K.K.; Duraiswamy, K.; Connolly, J.D. *Phytochemistry* **1984**, *23*, 135.
7. Jarvis, A.P. *Isolation and degradation of triterpenoids from tissue cultures and seeds of neem (Azadirachta indica).* Ph.D. Thesis, Keele University, 1998; p 97.
8. Garg, H.S.; Bhakuni, D.S. *Phytochemistry* **1984**, *23*, 2383.
9. Thiele, S. *Isolierung und Strukturaufklärung neuer Tetranortriterpenoide aus Azadirachta indica A. Juss. (neem tree, Meliaceae) und Untersuchung der nematiziden Wirkung von Neem Extracten.* Ph. D. dissertation, University of Hohenheim, 1991.
10. Ara, I.; Siddiqui, B.S.; Faizi, S.; Siddiqui, S. *Phytochemistry* **1988**, *27*, 1801.
11. Jarvis, A.P.; Johnson, S.; Morgan, E.D.; Simmonds, M.S.J.; Blaney, W.M. *J. Chem. Ecol.* **1997**, *23*, 2841.
12. Kernan, M.R.; Faulkner, D. J. *J. Org. Chem.*, **1988**, *53*, 2773.
13. Graziano, M.L.; Iesce, M.R.; Cinotti, A.; Scarpati, R. *J. Chem. Soc., Perkin Trans. I* **1987**, 1833.
14. Rojatkar, S.R.; Joshi, S.P.; Nagasampagi, B.A. In *Neem and Environment*, Singh, R.P.; Chari, Raheja, A.K.; Kraus, W., Eds; Science Publishers: Lebanon NH, 1996; Vol. 1.p 199.
15. Johnson, S.; Morgan, E.D. *J. Chromatogr. A* **1997**, *761*, 53.