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Photodegradation of azadirachtin-A: A neem-based pesticide

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Azadirachtin-A when exposed to UV light (254 nm), as a solid thin film on a glass surface, furnished only a single photoproduct. The photoproduct was isolated by repeated column chromatography and identified by NMR and mass spectroscopy. NMR data indicated that the (E)-2-methylbut-2-enoate ester group of azadirachtin-A has been converted into (Z)-2-methylbut-2-enoate ester. Half-life of azadirachtin-A as thin film under UV light was found to be 48 min.

NEEM plants, like all plants, have several different active chemical compounds. One group of compounds called limonoids, are of special interest. More specifically, one of these limonoids, which is unique to the neem plant, is called azadirachtin and is found in abundance in neem extracts. Most of the research that has been done on neem plant has focused intensely on this compound because, it is the principal active ingredient of a unique insecticide which is thought to be the most useful and most fascinating by-product of the neem tree. It is thought to be so peculiar because of its properties as a bio-pesticide: it does not immediately kill the insect like most pesticides do. Instead, when an insect eats azadirachtin, it actively attacks the insect's reproductive cycle, its feeding pattern, its bodily development, as well as acting as a direct toxin. Thus, when azadirachtin is sprayed on the plant and the insect takes a bite – if it can stand the bitter taste – the insect will no longer be able to reproduce, eat or grow. This unique compound only affects the insects that consume it, other 'friendly' insects which may help pollination and other plant functions are not harmed; it is a 'narrow spectrum pesticide'. Tests have shown azadirachtin to be effective on at least 200 different insect species[1]. Limonoid compounds are found in almost all parts of the neem plant. The active ingredients in neem extracts are very low in toxicity and thus are not toxic to mammals and also quickly biodegrade from the sun's light.

Azadirachtin-A, a powerful insect antifeedant and growth-regulating substance with exceptional environmental characteristics, has been isolated from the seed kernels of the neem tree (*Azadirachta indica* A. Juss). However, the use of azadirachtin-based neem pesticides may be limited by the acid and base sensitivity of the compound and its susceptibility to photodegradation due to presence of light-absorbing moieties[2,3]. The (E)-2-methylbut-2-enoate ether group of azadirachtin-A has been converted into (Z)-2-methylbut-2-enoate[4] in the photoreaction of azadirachtin-A in benzene solution, under both nitrogen and oxygen atmosphere. An alcohol adduct across the double bond was obtained when azadirachtin-A was exposed to UV light in alcohol[5]. There are no reports on the isolation of photoproduct on irradiation of azadirachtin-A as thin film on glass surface. Therefore, in this paper we report the isolation and identification of a photoproduct on irradiation of azadirachtin-A as a thin film on glass surface.

Azadirachtin-A was isolated and purified from neem (*A. indica*) seeds using a previously published method[6]. It was further purified by crystallization from carbon tetrachloride to a melting point of 149–150°C. The purity was checked by high-pressure liquid chromatography. A sample of analytical azadirachtin-A was supplied by SPIC Foundation.

For studies on glass surface as a thin film, a solution of azadirachtin-A in acetone (1 mg ml^{-1}) was applied uniformly with a pipette to petri plates (5 × 5 cm dia.). Acetone was evaporated at room temperature, leaving

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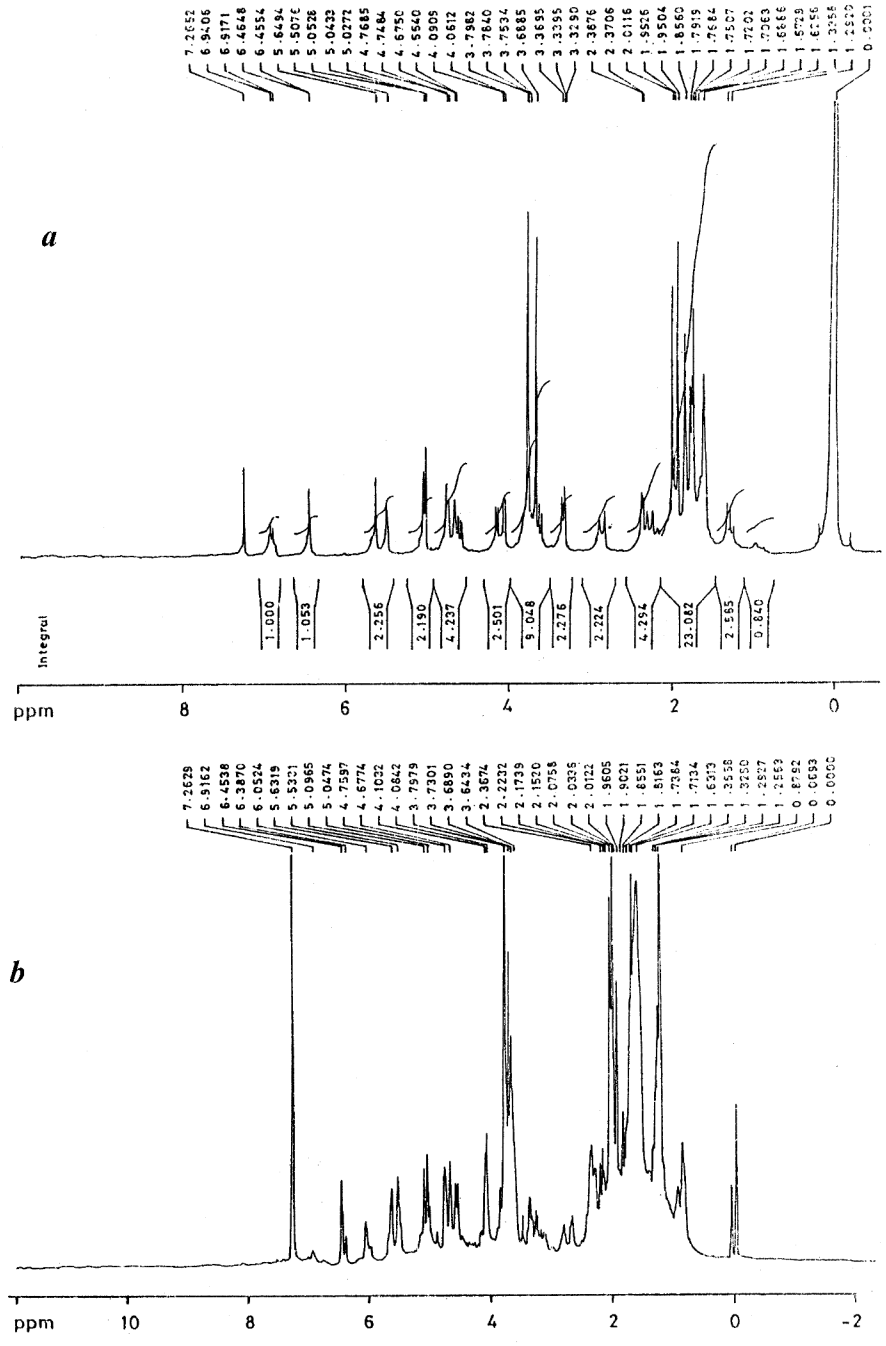

Figure 1. Proton NMR spectra of (*a*) Azadirachtin-A and (*b*) its photoproduct.

behind a thin layer of azadirachtin-A. These plates were exposed to UV light by placing them under an UV lamp at a distance of 30 cm (light produced by the lamp was rich in irradiation at 254 nm). After irradiation for 1 h, when there was no further conversion of azadirachtin-A to photoproduct (40:60 ratio), these plates were extracted with acetone (3 × 15 ml). To produce enough of the photoproduct for structural analysis, 200 mg of azadirachtin was irradiated. The combined extracts were concentrated and photoproduct was separated by column chromatography using a glass column (75 cm × 2 cm id) containing 50 g of preactivated 200–300 mesh silica gel in hexane, eluting with methylene chloride and acetone in different ratios. The methylene chloride: acetone (93:7 v/v) fraction gave a colourless solid, which crystallized from carbon tetrachloride (10 mg). Thin layer chromatography (TLC) of this fraction was performed on 20 cm × 20 cm glass plates coated with

Table 1. [^{1}H] NMR spectral data of azadirachtin-A and photoproduct

Proton	Azadirachtin-A	Photoproduct
H-1	4.76 (dd)	4.75 (ddd)
H-2	2.15 (ddd)	2.17 (ddd)
H-2	2.31 (ddd)	2.31 (ddd)
H-3	5.50 (dd)	5.53 (dd)
H-5	3.37 (d)	3.36 (dd)
H-6	4.58 (d)	4.58 (d)
H-7	4.76 (d)	4.75 (d)
H-9	3.33 (s)	3.33 (s)
H-15	4.67 (d)	4.67 (d)
H-16a	1.75 (ddd)	1.73 (ddd)
H-16b	1.33 (d)	1.32 (d)
H-17	2.38 (d)	2.36 (d)
H-18	2.01 (s)	2.01 (s)
H-19a	3.64 (d)	3.64 (d)
H-19b	4.17 (d)	4.16 (d)
H-21	5.64 (s)	5.63 (s)
H-22	5.05 (d)	5.04 (d)
H-23	6.46 (d)	6.45 (d)
H-28a	3.75 (d)	3.73 (d)
H-28b	4.09 (d)	4.08 (d)
H-30	1.75 (s)	1.73 (s)
7-OH	2.84 (br s)	2.68 (br s)
11-OH	5.04 (s)	5.04 (s)
20-OH	2.90 (s)	2.81 (s)
12-OMe	3.68 (s)	3.68 (s)
29-OMe	3.79 (s)	3.79 (s)
OAc	1.95 (s)	1.96 (s)
Tigloyl proton		
H-3′	6.94 (qq)	5.99 (qq)
H-4′	1.79 (dq)	1.81 (dq)
H-5′	1.85 (dq)	1.85 (dq)

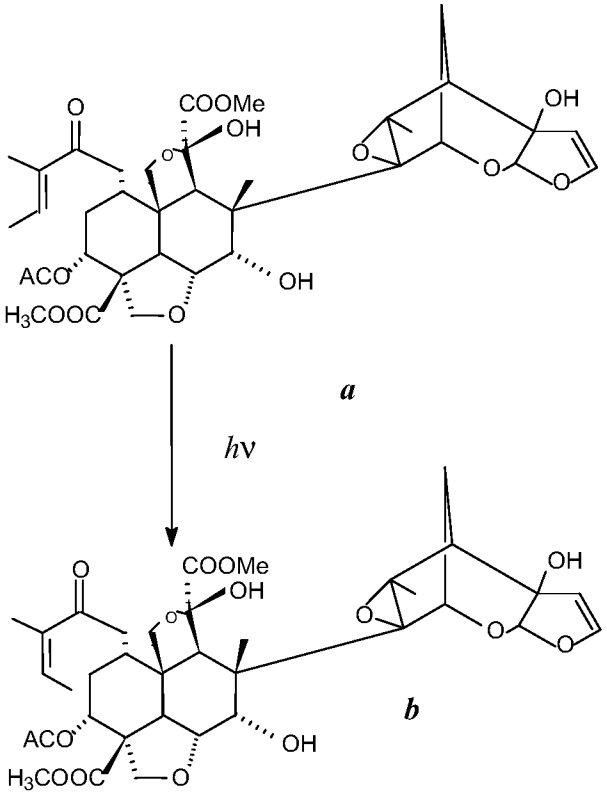

Figure 2. Structure of (*a*) azadirachtin-A and (*b*) photoproduct.

silica gel G (0.25 mm) using mobile phase as benzene + ethylacetate (1:1 by volume). Spraying with a chromogenic solution of vanillin and sulphuric acid identified the spots. The Rf of azadirachtin-A and the colourless solid was 0.54 and 0.62, respectively. Elution of column gave 10 mg of pure photoproduct, along with azadirachtin (5 mg), mixture of photoproduct and azadirachtin (20 mg) and some very polar products which could not be separated.

[^{1}H] NMR spectrum was recorded on a Brucker 400 MHz NMR instrument. Deuterochloroform ($CDCl_3$) was used as a solvent and tetramethylsilane (TMS) as the internal standard for NMR. FAB.MS was done on a Varian MAT 112S mass spectrometer in the positive ion mode.

The [^{1}H] NMR spectral data for photoproduct and azadirachtin-A are given in Table 1 and Figure 1. When [^{1}H] NMR spectra of azadirachtin-A and photoproduct were compared, it was found that the signals due to H-1 to H-23 protons (Table 1) of azadirachtin-A molecule are also present at the same fields without any shift in the spectrum of the photoproduct. But an upfield shift by δ 0.85 was observed in the tigloyl proton in the photoproduct. The tigloyl proton which is present at δ 6.94 (qq) in azadirachtin-A, was observed at δ 5.99 in the photoproduct (Table 1 and Figure 1). This shift was thought to be the result of isomerizing the [E]-2-methylbut-2-enoate group to the [Z]-2-methylbut-2-enoate group (Figure 2). The structure of the photoproduct was further confirmed by its FAB-MS spectrum, which showed molecular ion peak at m/z 721 (MH^+), which is similar to the FAB-MS of azadirachtin-A.

The structure was further confirmed by comparing the [^{1}H] NMR spectrum of the photoproduct with the photoproduct reported earlier[4], by irradiating azadirachtin-A in benzene solution. It can be concluded from the above observation that azadirachtin-A is highly photolabile and undergoes isomerization of (E)-2-methylbut-2-enoate ester group into (Z)-2-methylbut-2-enoate ester of azadirachtin-A, when irradiated as a thin film under UV light at 254 nm, due to the presence of π-electrons in the tigloyl moiety.

It was interesting to note that when azadirachtin-A was irradiated with UV light of 300–360 nm, the isomerized product was not formed. Thus it can be postulated that the [E]-2-methylbut-2-enoate group of azadirachtin-A absorbs in the UV at a slightly shorter

wavelength (254 nm), a non-planar transition state is obtained and returned to that ground state possessing the lowest energy. Since the *trans* isomers usually are of lower energy than the *cis* isomer, isomerization leads to a mixture in which the *trans* isomer generally predominates. Furthermore, it was observed that continued irradiation of azadirachtin-A with UV light led to a point where the *cis*/*trans* ratio (40:60) in the mixture no longer altered, because a constant ratio of the excited species is being generated, leading to a constant ratio of isomers on decay.

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Association of a tospovirus with sunflower necrosis disease

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Association of a tospovirus with necrosis disease-affected sunflower samples collected from Bangalore, Dharwad and Hyderabad was established on the basis of immuno- and bio-diagnosis. The virus induces necrosis on leaves followed by necrosis on petioles, stems and floral calyx. The virus was mechanically transmitted to cowpea, a diagnostic assay host for tospovirus. The sunflower tospovirus, tentatively referred to as sunflower necrosis virus (SFNV), showed positive serological relationship with groundnut bud necrosis virus (GBNV) and watermelon silver mottle virus (WSMV) in direct antigen-coated (DAC)-ELISA using polyclonal antisera directed against nucleocapsid protein. The exact identity of the virus remains to be determined.

A large number of viruses belonging to cucumo-, ilar-, poty-, tospo- and umbra-virus groups have been recorded on sunflower[1,2]. In India, only the association of a potyvirus has been established with mosaic-affected sunflower samples collected from Delhi and Hyderabad[3]. An unusual necrosis disease on sunflower was observed in serious proportion in parts of Karnataka during the summer in 1997 (ref. 4). Incidence of this disease up to 80% on a breeding line (CMS234) was recorded in 1999 during a survey of sunflower growing areas in Karnataka[5]. Since then the disease became increasingly important in sunflower-growing areas of Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu. The disease is characterized by necrosis on leaves followed by necrosis on petioles, stems and floral calyx (Figure 1 *a* and *b*). In some genotypes the leaves develop typical mosaic, rings and line pattern. Early infected plants remain stunted and develop malformed heads with poor or no seed setting, resulting in complete loss of the crop. Though the disease has been noticed in severe form both on traditional genotypes as well as hybrids since 1997, its etiology has still not been worked out. In this paper we report our studies on the etiology which revealed the association of a tospovirus with the sunflower necrosis disease, based on immuno- and bio-diagnosis.

A total of 22 sunflower necrosis diseased samples collected from Bangalore, Dharwad and Hyderabad during different periods were assessed for the presence of virus (Table 1). Standard direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA)[6] was performed using antisera to cucumo-, poty- and tospoviruses. Polyclonal antisera to groundnut chlorotic fleck virus (GCFV), groundnut ring spot virus (GRSV), impatiens necrotic spot virus (INSV), tomato chlorotic spot virus (TCSV) and tomato spotted wilt virus (TSWV) obtained from A. C. de Avila, EMBRAPA, Brazil, antiserum to watermelon silver mottle virus (WSMV) from S. D. Yeh, National Chung Hsing University, Taichung, Taiwan and antiserum to groundnut bud necrosis virus (GBNV) from D. V. R. Reddy, ICRISAT, India were used. Polyclonal antisera to cucumber mosaic virus (CMV) and potato Y virus (PVY) were ob-

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