Transformation of Azadiradione to Nimbocinol and 17β-Hydroxynimbocinol, and Structure–Pesticidal-Activity Relationship of Triterpenoids isolated from *Azadirachta indica* A. Juss. (Neem)

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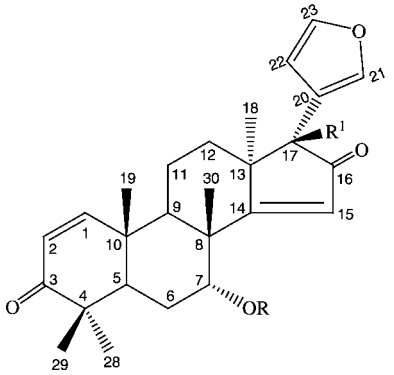
During studies on the structure–pesticidal-activity relationship of *Azadirachta indica* constituents, azadiradione (**1**) was treated with methanolic K_2CO_3 to obtain 7-deacetyl derivative nimbocinol (**2**). Unexpectedly, 17β-hydroxynimbocinol (**3**), reported earlier as a natural product, was also formed. The structure–pesticidal-activity relationship of triterpenoids **1**–**16** against *Anopheles stephensi* Liston is described towards understanding the active functionalities responsible for the pesticidal activity of triterpenoids having apoeuphane(apotirucallane) and gedunin skeleta. The pesticidal activities of triterpenoids **2**–**8** are reported for the first time.

1. Introduction. – *Azadirachta indica* A. Juss. (Syn. *Melia indica* Brandis; *Melia azadirachta* Linn.), known commonly as neem (Urdu) and Indian lilac (English), belongs to the Meliaceae family (order Rutales). Neem, a tropical to subtropical plant, is native to the southern part of Asia, particularly Southeast Asia, including Pakistan. It was introduced in Africa in the beginning of the last century and is now widely cultivated in more than 30 countries, particularly along the Sahara's southern fringe. Over the last century or so, plantations of the tree have also been established in Fiji, Mauritius, the Caribbean and many countries of Central and South America and the USA. The tree, with an average lifespan of more than 200 years, starts fruiting after 3 to 5 years and can produce up to 50 kg of fruit annually. The ripe, ellipsoidal drupe (fruit) measures up to almost 2 cm in length, comprising sweet pulp and a seed covered in a smooth yellow or yellowish-green coat [1].

Medicinal uses of various parts of neem are manifold and have been particularly ascribed to its leaves, fruit, oil, and bark. Its different parts are highly reputed in folklore and traditional medicine for the treatment of a variety of human ailments and have been inherited as a tradition in the Indo-Pak culture [2][3]. Neem has been studied for several decades for insecticidal and insect-repellant properties and the chemical constituents responsible [1–10]. Since the report of azadirachtin, perhaps the most outstanding potent, naturally occurring pesticide [11], scientists have begun to undertake systematic chemical analyses to isolate and identify the biologically active constituents, monitoring their entomological properties and have found that neem botanicals can affect a broad spectrum of pests, including insects, nematodes, fungi,

bacteria, and even a few viruses. Unlike ordinary insecticides based on a single active ingredient, neem botanicals contain several complex arrays of novel, active constituents that collectively act in more than one way to produce abnormal insect development and molting, hormonal disturbances, growth inhibition, antifertility, antifeeding, and oviposition, but not as outright killers [1][4–6][9][10].

Chemical studies of neem's active principles have led to the isolation of a host of chemical constituents, characterization and structure–activity-relationship studies of rearranged and modified azadirachtins against *Spodoptera littoralis* [12][13], synthetic analogues of azadiradione (**1**) [14] and epoxyazadiradione (**9**) [15], and other limonoids [16–18].



R = Ac, R^1 = H Azadiradione (**1**)
R = Ac, R^1 = OH, 17β-Hydroxyazadiradione
R = H, R^1 = H Nimbocinol (**2**)
R = H, R^1 = OH 17β-Hydroxynimbocinol (**3**)

Anopheles stephensi Liston, a malarial-parasite-carrier mosquito was used in the present study as it is the second-most-common anopheline in this region [19][20], where malaria has been estimated to represent 2.3% of the overall global-disease burden [21].

K_2CO_3 is ideal for selective deprotonation of acids and, in combination with various solvents, has been used by investigators for the alkylation of phenols and 1,3-dicarbonyl compounds, deamination of β-amino esters to the corresponding alcohols, and epoxidation of amides with an α,β-aminoalcohol moiety, and to obtain intramolecular *Michael* adducts [22]. Methanolic K_2CO_3 has been used for the detigloylation of analogues obtained in oxidative studies on 11-acetoxy-22,23-dihydroazadirachtin [23] and to convert allylic esters to the corresponding alcohols [24].

2. Results and Discussion. – Systematic investigation of the ethanolic extract of the fruit coat [25][26] following a pesticidal-activity-guided isolation, with monitoring of *An. stephensi* Liston mortality, led to the isolation of five known triterpenoids, azadirone (**4**) [27], deoxygedunin (**5**) [28], gedunin (**6**) [28], α-nimolactone (**7**) [29], and β-nimolactone (**8**) [29], and the identification of various other natural products [30]. The pesticidal activity (LC_{50}) of these constituents determined against fourth-

instar larvae of *An. stephensi* by the WHO method is compared with that of azadiradione (**1**), epoxyazadiradione (**9**), desfuranoazadiradione (**10**), meliacinin (**11**), azadironic acid (**12**), limocin A and B (**13** and **14**), isolated earlier from fruit coats [25], and desfurano-6α-hydroxyazadiradione (**15**) and 22,23-dihydronimocinol (**16**) isolated from leaves [31] towards gaining insight into the effect of structure on pesticidal activity. The availability of these triterpenoids, each having the basic apoeuphane-(apotirucallane) or gedunin skleton, provided us with an opportunity to understand the active phores. To extend these studies to other analogues, **1** was hydrolyzed with methanolic K_2CO_3, which afforded nimbocinol (**2**) [14][32] along with 17β-hydroxy-nimbocinol (**3**), both of which are previously reported natural products [14][33].

A complete assignment of ^{1}H and ^{13}C nuclei of **3** has been made through spectroscopic methods, including ^{1}H- and ^{13}C-NMR (BB and DEPT), *J*-resolved, ^{1}H-^{13}C COSY, COSY-45°, NOESY, HMQC, HMBC (*Fig. 1*), which has led to the reassignment of some of the nuclei, including H–C(5), H$_{\alpha/\beta}$–C(6), H–C(9), H$_{\alpha/\beta}$–C(11), H$_{\alpha/\beta}$–C(12), H–C(21), H–C(23), C(5), and C(9) (*Table 1*). The known compounds have been identified through comparison of their physical and spectral data with those reported in the literature [27–29][34][35].

Formation of 17β-Hydroxynimbocinol (**3**). – The formation of **3** can be inferred on the basis of the acidity of H–C(17), which is also evident from the epimerization at C(17) *via* enolization of 16-keto analogues in strong alkaline medium, *e.g.*, in the formation of 17-epinimbocinol [14]. Although methanolic K_2CO_3 is not strong enough to cause complete enolization, it can readily oxidize the substrate carrying acidic protons with O_2 (analogous to 2-nitropropane, which autoxidizes in weakly but not strongly basic solution). Auto-oxidation reactions are slow, and some of them seem to proceed in the absence of the photosensitizer that would be required by classical theory for the generation of singlet oxygen ($^1O_2$) [36][37]. Such free-radical reactions, called electron-detachment- and -attachment reactions (ED/EA in the *Scheme*), are more-favorable when the radical is formed at a tertiary C-atom, since the relative reactivity for abstraction of H-atoms from tertiary aliphatic C-atoms is generally 4–6 times higher than that from secondary aliphatic C-atoms. In the present case, electron delocalization in the furan ring may stabilize the radical formed. Further, conformational strain contributes to retention of the α-orientation of furan [38][39].

Structure–Pesticidal-Activity Relationships. – Neem constituents represent a variety of structural diversity with varying order of biological activities. In the present studies, their activities against *An. stephensi* were compared. The main triterpenoids (tetranortriterpenoids) azadiradione (**1**), azadirone (**4**), and epoxyazadiradione (**9**), *e.g.*, possess a common ring A with 1-en-3-one system, an acetoxy substituent at C(7), and a furan ring at C(17), but vary in ring D. The above compounds display LC_{50} values of 15, 10, and 18 ppm, respectively (*Fig. 2*).

However, in gedunin (**6**), in which ring D is cleaved, the toxicity decreases to LC_{50} 120 ppm. The same trend is observed in deoxygedunin (**5**; LC_{50} 150 ppm), which also has a cleaved ring D. The furan ring also contributes to the toxicity of neem compounds, as could be inferred from the diminished toxicity of desfurano compounds relative to azadiradione, like in α-nimolactone (**7**; LC_{50} 60 ppm), β-nimolactone (**8**; LC_{50} 45 ppm), desfuranoazadiradione (**10**; LC_{50} 37 ppm), and desfurano-6α-hydroxyazadiradione (**15**; LC_{50} 43 ppm), as well as the 22,23-dihydronimocinol (**16**; LC_{50} 60 ppm).

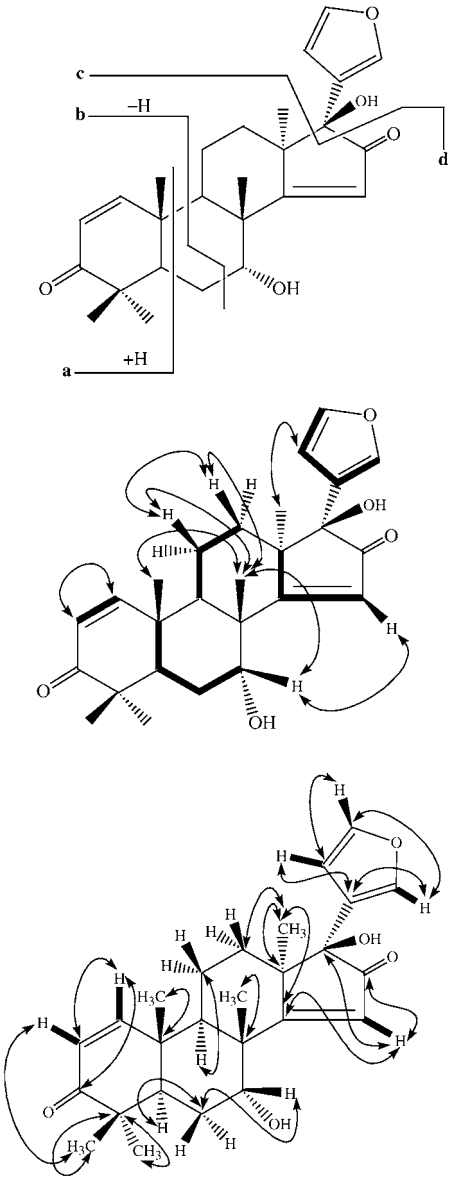


Fig. 1. *Spectroscopic data of* **3**. *Top:* Mass fragmentation pattern; *middle:* COSY-45° (bold lines), and NOE correlations (arrows); *bottom:* HMBC (arrows) and HMQC (bold lines).

Oxidation of the furan ring to an acetal ring on the other hand, does not give rise to any considerable alteration in activity (*e.g.*, limocin A and B (**13** and **14**): LC_{50} 19 ppm).

A further observation is that the presence of an α,β-unsaturated open-chain carbonyl moiety or double bond ($\Delta^{20,22}$) in an open chain increases the activity

Table 1. *^{1}H-,^{13}C-, and ^{1}H,^{1}H-COSY-45° Data* ($CDCl_3$) *of* **3**. Literature data of 17β-hydroxyazadiradione [34][35] included for comparison. Chemical shifts (δ) in ppm, coupling constants (J) in Hz.

	3			17β-Hydroxyazadiradione	
	δ(H)	δ(C)	COSY-45°	δ(H)	δ(C)
H–C(1)	7.11 (d, J = 10.2)	157.5	5.83	7.16 (d, J = 10.0)	157.15
H–C(2)	5.83 (d, J = 10.2)	125.7	7.11	5.89 (d, J = 10.0)	125.82
C(3)	–	204.7	–		204.11
C(4)	–	44.1	–		44.07
H_α–C(5)	2.42 (J = 13.0, 2.4)	44.4	1.99	2.20	38.81
H_α–C(6)	1.83 (J = 15.3, 2.7, 2.4)	25.5		2.12–1.55	22.39
H_β–C(6)	1.99 (J = 15.3, 13.0, 2.7)	25.5		2.12–1.55	–
H_β–C(7)	4.16 (J = 2.7, 2.7)	71.7	1.83, 1.99, 2.42	5.33	74.20
C(8)	–	46.8		–	44.85
H_α–C(9)	2.35 (J = 12.1, 6.7)	36.9	1.80	2.24	46.02
C(10)	–	40.2		–	39.95
H_α–C(11)	2.03 (J = 13.8, 9.6, 6.7, 3.4)	15.6	1.58, 1.80	2.12–1.55	15.89
H_β–C(11)	1.80 (J = 13.8, 12.1, 9.7, 3.2)	15.6	1.58, 1.80	2.12–1.55	–
H_α–C(12)	1.58 (J = 13.8, 9.7, 3.4)	22.2	1.80, 2.52, 1.58, 1.80, 2.03	2.12–1.55	23.50
H_β–C(12)	2.52 (J = 13.8, 9.6, 3.2)	22.2	1.80, 2.52, 1.58, 1.80, 2.03	2.12–1.55	–
C(13)	–	50.4		–	50.36
C(14)	–	195.4		–	193.42
C(15)	5.87 (s)	120.2		5.78	120.33
C(16)	–	206.2		–	205.84
C(17)	–	80.7		–	80.67
Me(18)	0.93 (s)	30.2	2.52	0.96 (s)	30.84
Me(19)	1.19 (s)	19.1		1.23 (s)	19.08
C(20)	–	122.5		–	122.57
H–C(21)	7.54 (dd, J = 1.84, 0.9)	141.4	7.38, 6.37	7.58	142.62
H–C(22)	6.37 (dd, J = 1.69, 0.9)	109.5	7.38, 7.54	6.39	109.63
H–C(23)	7.38 (dd, J = 1.84, 1.69)	142.8	6.37, 7.54	7.42	141.42
Me(28)	1.08 (s)	21.4		1.09 (s)	21.22
Me(29)	1.13 (s)	27.1		1.07 (s)	27.01
Me(30)	1.27 (s)	25.5		1.35	25.16
C(1′)	–	–		–	169.69
Me(2′)	–	–		1.94	20.96
17β-O*H*	3.43 (s)[a])	–			–

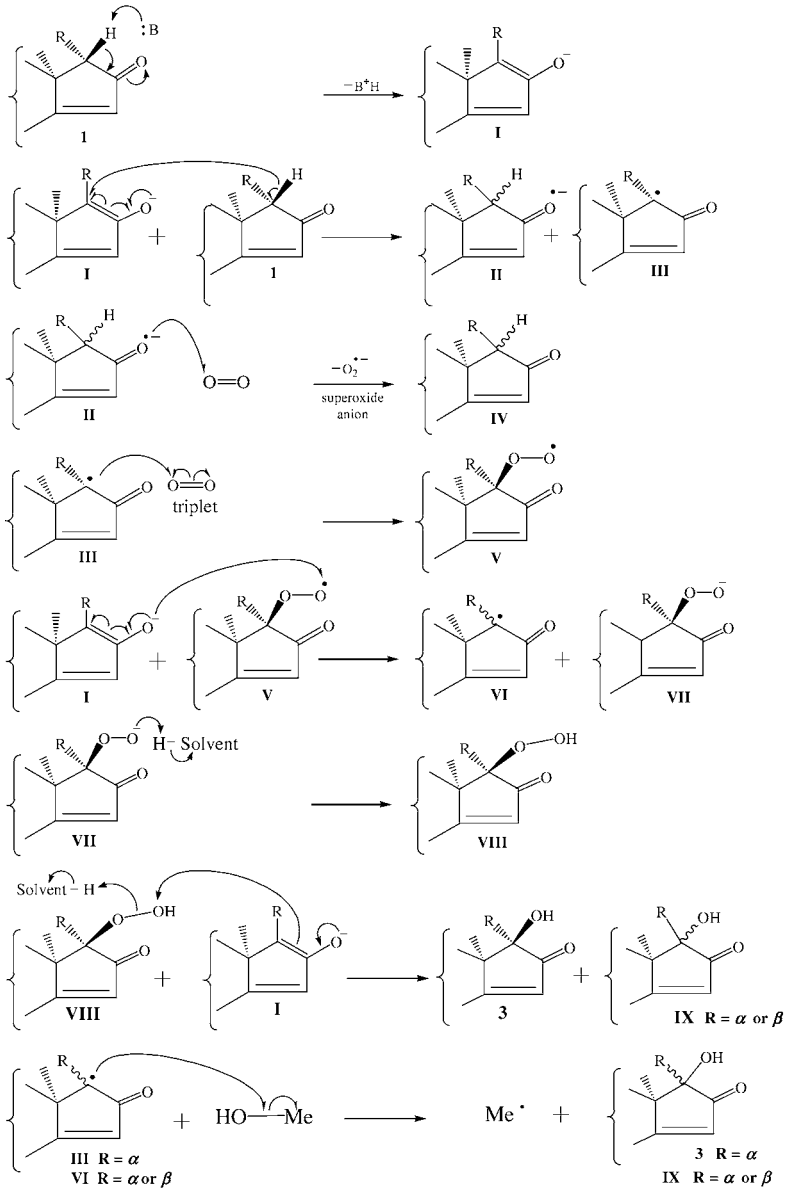
[a]) Exchangeable with D_2O.

significantly. This was exhibited by meliacinin (**11**; LC_{50} 13 ppm, with C=C in the side chain at C(17)), azadironic acid (**12**; LC_{50} 4.5 ppm, with α,β-unsaturated carbonyl side chain at C(17)), and its methyl derivative (**12a**; LC_{50} 2.8 ppm).

Moreover, deacetylation at C(7) decreases the activity as noted in nimbocinol (7α-deacetylazadiradione, **2**; LC_{50} 30 ppm). Hydroxylation at C(17), on the other hand (7α-deacetyl-7α,17β-dihydroxyazadiradione, **3**; LC_{50} 15 ppm), results in increased activity, identical to that of **1** (*Fig. 2*). This relationship of the activities in compounds **1**, **2**, and **3** is analogous to earlier observations of pesticidal activity against *Heliothis virescens* [33].

Replacement of the OH group by a MeO group at C(17) does not alter the activity notably, as shown by **11** (17β-OMe) and **3** (17β-OH), and the same can be inferred from

Scheme. *Proposed ED/EA Pathway for Oxidation of* **1** *to* **3** (D-ring)



15 and **16**, which have an α-hydroxy substituent at C(6). These studies also reflect that the right-hand part of the skeleton make an important contribution to the activity of these limonoids, as alterations brought about in this part exhibit a notable change in the order of activity.

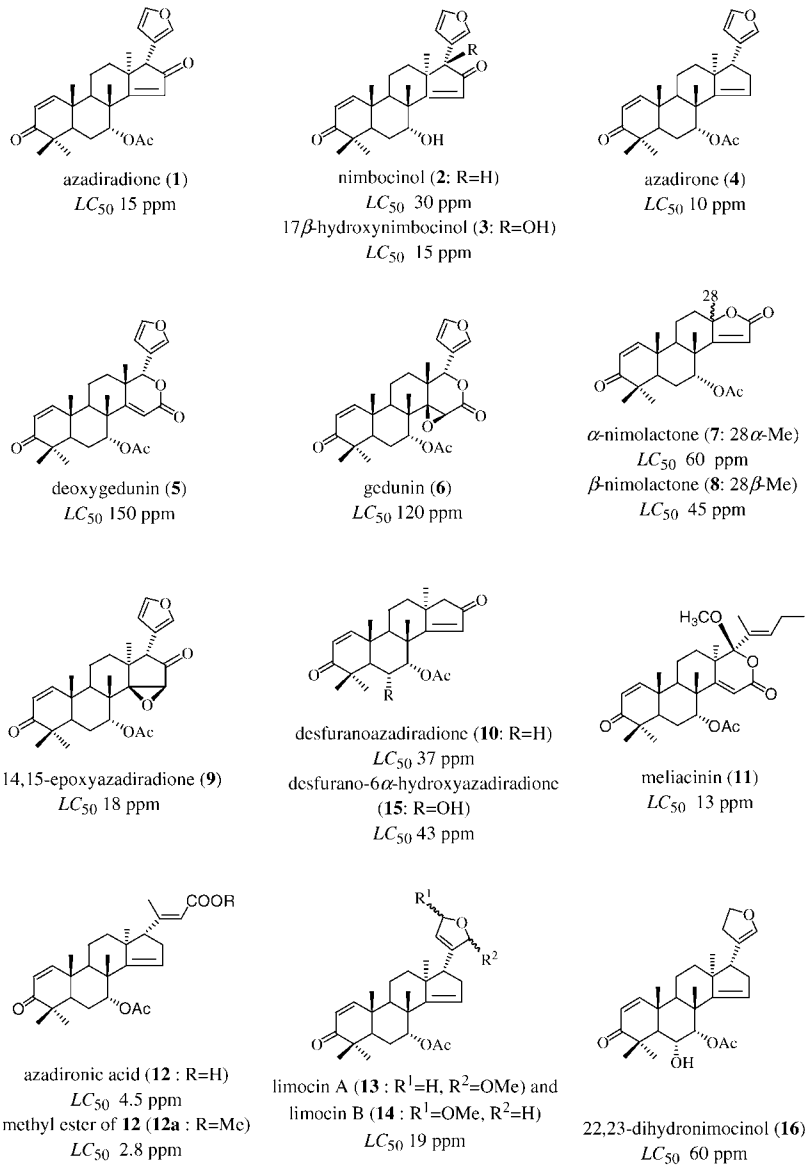


Fig. 2. *Triterpenoids* **1**–**16**: *structures and* LC_{50} *values for pesticidal activity against* An. stephensi

A synthetic pesticide of pyrethroid class, permethrin (*Coopex® EC* 2.5%) was also assayed for pesticidal activity in parallel. Its LC_{50} value against fourth instar larvae of *An. stephensi* was 0.125 ppm.

Experimental Part

General. UV spectra: MeOH solns.; *Hitachi U-3200* spectrophotometer; λ_{max} in nm. FT-IR-ATR (Attenuated Total Reflectance) Spectra: $CHCl_3$ solns.; *Bruker Vector-2000-FTIR* spectrophotometer, with Opus (Version 3.0) software; ν in cm^{-1}. ^{1}H-NMR Spectra: $CDCl_3$ solns.; *Bruker Avance-400* spectrometer at 400 MHz; ^{13}C-NMR, DEPT, COSY-45°, NOESY, *J*-resolved, HMQC, and HMBC experiments: *Bruker Avance-500* spectrometer at 500 MHz; δ in ppm rel. to $SiMe_4$, *J* in Hz. ^{1}H-^{13}C COSY Spectra: *Bruker Aspect-300* instrument at 300 MHz. Spectral assignments made on the basis of combined spectroscopic data. LR-EI-MS and HR-EI-MS; double-focussing *Finnigan MAT-312* and *Jeol JMS-HX-110* mass spectrometers, resp.; ion source at 250° and 70 eV; *m/z* (rel. %). Elemental analyses: *Carlo-Erba 1106* instrument.

Anal. TLC and prep. thin-layer chromatography (prep. TLC): *Merck Kieselgel 60 PF_{254}* coated on glass plates; detection by UV illumination (254 and 366 nm) and by iodine spray. Vacuum liquid chromatography (VLC): *Merck Kieselgel 60*. Flash column chromatography (FC): *Eyela* model *EF-10* or *Aldrich* flash column chromatograph; *Merck 9385* silica gel, 0.040–0.063 mm. Prep. HPLC: *Shimadzu LC-8A*; *UV SPD-6A* detector (at 254 nm); *Shimpack PREP ODS* column (No. 2025752, 250 mm × 22 mm i.d.; *Shimadzu*, Kyoto, Japan); mobile phase 50–90% aq. MeOH; flow rate 50–120 ml/min in steps.

Bioassay-Guided Extraction and Isolation. Plant material and bioassay-guided extraction and isolation of compounds has been described for the thirteen VLC fractions 'A' to 'M' in a previous communication [25].

Fractions 'A' to 'C' were found to contain constituents other than triterpenoids [30]. The mother liquor of 'D' after crystallization of **9**, on prep. TLC (silica gel, petroleum ether/AcOEt 70:30, 254 nm) afforded azadirone [27] (**4**; 26 mg). Fractions 'E' afforded **1** and **9** in equal amounts. Fractions 'F' (petroleum ether/AcOEt 50:50 (2) eluate) and 'G' [petroleum ether/AcOEt, 40:60 (2) eluate] were combined, dissolved in a minimum quantity of AcOEt and poured into excess petroleum ether to precipitate limonoids. The clear supernatant that separated on standing overnight was decanted, and the insoluble residue separated by prep. TLC (silica gel, petroleum ether/AcOEt 60:40) to afford **1**, **9**, and **5** [28]. Fractions 'D' through 'G', showing one or two major spots on anal. TLC were further purified to give compounds **1**, **4**, **5**, and **9**. The pesticidal activities of **1** and **9** have been reported earlier [25], while those of **4** and **5** are reported in the present communication.

Fraction 'L' (1.53 g), the most-active fraction, was subjected to FC to afford 16 fractions (LFC-1–LFC-16). Three of the fractions eluted with a gradient from $CHCl_3$/MeOH 99:1 to $CHCl_3$/MeOH 95.5:4.5 (elution vol., 1600 ml), *i.e.*, fractions 'LFC-6' (181.9 mg; LC_{50} 23.5 ppm) and 'LFC-7' (41 mg; LC_{50} 25.3 ppm), and 'LFC-8' (70.2 mg; LC_{50} 19.2 ppm) were combined to give 293.1 mg, that was resubmitted to FC on an Aldrich chromatograph (silica gel, $CHCl_3$, $CHCl_3$/MeOH, and MeOH in order of increasing polarity). The fractions were finally combined on the basis of TLC to give 22 fractions, 'LFC-601' to 'LFC-622'.

Fraction 'LFC-611' (27 mg), which was eluted with $CHCl_3$/MeOH, 99:1 (elution vol. 750 ml), on further purification by prep. TLC, afforded pure gedunin [28] (**6**; 22 mg; LC_{50} 120 ppm). Fraction 'LFC-618' (38 mg), which eluted with $CHCl_3$/MeOH, 96:4 (elution vol. 250 ml) and 95:5 (elution vol. 500 ml) furnished pure α-nimolactone [29] (**7**; 12 mg) and β-nimolactone [29] (**8**; 16 mg) on prep. TLC (silica gel, $CHCl_3$ with a few drops of MeOH)].

17β-Hydroxynimbocinol (=*21,23-Epoxy-7α,17β-dihydroxy-24,25,26,27-tetranorapotirucalla-1,14,20,22-tetraene-3,16-dione*; **3**). *Hydrolysis of* **1**: Azadiradione (**1**; 100 mg, 0.222 mmol) was dissolved in a minimum amount of MeOH. To this soln., a super-sat. soln. of methanolic K_2CO_3 (10 ml, along with residual insoluble alkali) [23][24] was added, and the mixture stirred vigorously at r.t. for 8 h. The reaction was monitored by anal. TLC for the disappearance of **1**, poured into cold sat. aq. NH_4Cl (20 ml), and extracted with AcOEt (3×). The AcOEt layer was dried (anh. Na_2SO_4), evaporated, and purified by reverse-phase prep. HPLC (RP-18, MeOH/H_2O 30:70) to furnish crystalline nimbocinol (deacetylazadiradione; **2**, 60 mg) and 17β-hydroxynimbocinol (**3**, 11.4 mg) in a ratio of 5.2:1.

Data of **3**: Needle-like crystals (11.4 mg). M.p.: 238° (dec.). $[\alpha]_D^{27}$: +54.84 (c = 0.248, MeOH). UV: 238. IR: 3600–3200 (OH), 1698, 1665 (RCH=CHCOOR, RCH=CHCOR), 1595 (C=C), 1454, 1386 (Me_2C), 1159, 1033 (C–O), 870 (trisubstituted C=C, furan). ^{1}H- and ^{13}C-NMR and COSY-45°: see *Table 1*. HR-EI-MS: 424.2251 (38, $C_{26}H_{32}O_5$, M^+), 409.1996 (28, $C_{25}H_{29}O_5^+$, $[M-Me]^+$), 406.2117 (3.5, $C_{26}H_{30}O_4^+$, $[M-H_2O]^+$), 391.1927 (5, $C_{25}H_{27}O_4^+$, $[M-Me-H_2O]^+$), 329.2117 (11, $C_{21}H_{29}O_3^+$, **d**), 229.1154 (2, $C_{15}H_{17}O_2^+$), 149.0978 (12, $C_{10}H_{13}O^+$, **a**), 137.0982 (12, $C_9H_{13}O^+$, **b**), 95.0150 (100, $C_5H_3O_2^+$, **c**) (*Fig. 1*). Anal. calc. for $C_{26}H_{32}O_5$ (424): C 73.6, H 7.6; found: C 71.8, H 8.6.

Pesticidal Activity: Rearing: The fourth-instar larvae of *An. stephensi* Liston (Orangi Town Wild Strain), a vector of the malarial parasite, were collected directly from the natural environment especially established for this research work. The size of the pond was 2.4 × 1.2 m with a depth of 0.6 m. The pupae from the pond were collected and kept in cages for hatching.

Preliminary Screening Procedure: Ten young fourth-instar larvae of *An. stephensi* were collected in 5 ml of tap water and transferred to a 100 ml glass beaker containing 45 ml of distilled water. The fractions were tested at $28 \pm 1°$ at five final concentrations. Controls were similarly prepared. Each concentration and control was run as a duplicate set, and mortality was recorded after 24 h. See *Table 2*.

Table 2. LC_{50} *Values Determined for Fractions from* A. indica *Fruit Coats and Seeds against Fourth-Instar Larvae of* An. stephensi

Fraction No.		LC_{50} [ppm]	Fraction No.		LC_{50} [ppm]
Main fractions:					
1	RB-b (Fresh fruit-coat extract)[a])	290	6	PE 'S' (Petroleum ether soluble fraction of RB-b 'N')	159
2	RB-a (Fresh seed extract)[a])	784	7	PE 'I' (Petroleum ether insoluble fraction of RB-b 'N')	154
3	AcOEt phase of FFC (RB-b) after liquid–liquid partitioning	165	8	'ES' (Ether soluble fraction of PE 'S')	106
4	RB-b 'A'[b]) (Acidic fraction of AcOEt phase)	144	9	'EI' (Ether insoluble fraction of PE 'I')[c])	15
5	RB-b 'N' (Neutral fraction of AcOEt phase)	43			
VLC-fractions from 'ES'					
10	'A'	100	15	'I'	107
11	'B'	200	16	'J'	136
12	'C'	150	17	'K'	212
13	'D'–'G'	[d])	18	'L'	66
14	'H'	215	19	'M'	182

[a]) The LC_{50} value determined with fourth-instar larvae of *Aedes aegypti* was found to be 319 ppm for RB-b and 446 ppm for RB-a. [b]) This extract, when tested after 6 months, gave a LC_{50} value of 300 ppm. [c]) Consisted exclusively of azadiradione (**2**). [d]) These fractions were mixtures of **1**, **4**, **5**, and **9**, and were, therefore, not assayed as such, although the activity of pure compounds was determined (see *Fig. 2*).

Mortality Testing: The *WHO* method [40] was modified for this application. A batch of ten fourth-instar larvae were released in a 100-ml beaker containing 50 ml filtered tap water. The conc. selected in the preliminary screening of each compound were tested at $28 \pm 1°$. A group of seven beakers was set up, five for the different concentrations of pesticide and one each for positive and negative controls. Each experiment was repeated five times. Experiments in which the mortality in the negative control was found to be more than 10% were discarded. The mortality was recorded after 24 h and readings were subjected to *Abbot*'s formula [41].

Calculation of LC_{50}*:* The lethal concentrations (LC_{50}) were calculated by means of the PROBIT analysis [42]. Both RB-b and RB-a were also tested for toxicity against *Aedes aegypti* L., and LC_{50} were found to be 319 and 446 ppm, resp. The detailed statistical data is presented in *Table 3*.

Table 3. *Statistical Analysis of Pesticidal Activity*

Entry	Dose [ppm]	Mean mortality [% ± SD][a])	Dose [ppm]	Mean mortality [% ± SD][a])	Dose [ppm]	Mean mortality [% ± SD][a])
	Main fractions:					
	RB-b		RB-a		EtOAc phase of FFC	
1	100.0	20 ± 7	400.0	22 ± 4.48	80.0	20 ± 7.07
2	200.0	34 ± 5	600.0	36 ± 5.48	120.0	34 ± 5.48
3	300.0	52 ± 4	800.0	54 ± 5.48	160.0	42 ± 4.48
4	400.0	82 ± 4	1000.0	72 ± 4.48	200.0	66 ± 5.48
5	500.0	98 ± 4	1200.0	96 ± 4.48	240.0	82 ± 4.48
	RB-b 'A'		RB-b 'N'		PE 'S'	
1	125.0	36 ± 5	20.0	8 ± 4	80.0	22 ± 4
2	150.0	54 ± 5	30.0	28 ± 4	120.0	36 ± 5
3	175.0	72 ± 4	40.0	52 ± 8	160.0	44 ± 5
4	200.0	84 ± 5	50.0	62 ± 4	200.0	72 ± 4
5	225.0	92 ± 4	60.0	76 ± 5	240.0	88 ± 4
	PE 'I'		ES		EI	
1	80.0	24 ± 5	75.0	44 ± 5	10.0	34 ± 5
2	120.0	36 ± 5	125.0	54 ± 5	15.0	50 ± 7
3	160.0	56 ± 5	175.0	66 ± 5	20.0	68 ± 4
4	200.0	74 ± 5	225.0	76 ± 5	25.0	84 ± 5
5	240.0	98 ± 4	275.0	88 ± 4	30.0	92 ± 4
	VLC-Fractions from 'ES':					
	Fraction 'A'		Fraction 'B'		Fraction 'C'	
1	55.5	8 ± 4	110.2	2 ± 4	92.2	14 ± 5
2	74.0	26 ± 5	147.0	8 ± 4	123.0	22 ± 4
3	92.5	48 ± 4	183.7	36 ± 5	153.7	52 ± 8
4	111.0	78 ± 4	220.5	56 ± 5	184.5	76 ± 5
5	229.5	92 ± 4	257.2	82 ± 8	215.2	94 ± 5
	Fraction 'H'		Fraction 'I'		Fraction 'J'	
1	50.0	8 ± 4	50.0	28 ± 4	50.0	24 ± 5
2	100.0	14 ± 5	100.0	48 ± 4	100.0	36 ± 5
3	150.0	36 ± 5	150.0	72 ± 4	150.0	56 ± 5
4	200.0	44 ± 5	200.0	88 ± 4	200.0	62 ± 4
5	250.0	66 ± 5	250.0	96 ± 4	250.0	88 ± 4
	Fraction 'K'		Fraction 'L'		Fraction 'M'	
1	50.0	8 ± 4	25.0	20 ± 7	50.0	22 ± 4
2	100.0	14 ± 5	50.0	44 ± 5	100.0	34 ± 5
3	150.0	34 ± 5	100.0	62 ± 4	150.0	42 ± 4
4	200.0	46 ± 5	150.0	76 ± 5	200.0	56 ± 5
5	250.0	62 ± 4	200.0	96 ± 4	250.0	76 ± 5
	Purified triterpenoids and permethrin					
	1		**2**		**3**	
1	10.0	34 ± 5	10.0	22 ± 4	10.0	36 ± 5
2	15.0	50 ± 7	20.0	36 ± 5	15.0	50 ± 7
3	20.0	68 ± 4	30.0	50 ± 7	20.0	72 ± 4
4	25.0	84 ± 5	40.0	76 ± 5	25.0	88 ± 4
5	30.0	92 ± 4	50.0	94 ± 5	30.0	98 ± 4

Table 3 (cont.)

Entry	Dose [ppm]	Mean mortality [% ± SD][a])	Dose [ppm]	Mean mortality [% ± SD][a])	Dose [ppm]	Mean mortality [% ± SD][a])
	4		**5**		**6**	
1	2.5	14 ± 5	50.0	14 ± 5	40.0	8 ± 4
2	5.0	28 ± 4	100.0	34 ± 5	80.0	28 ± 4
3	7.5	36 ± 5	150.0	50 ± 7	120.0	50 ± 7
4	10.0	50 ± 7	200.0	68 ± 4	160.0	72 ± 4
5	12.5	68 ± 4	250.0	88 ± 4	200.0	92 ± 4
	7		**8**		**9**	
1	20.0	20 ± 7	15.0	22 ± 4	13.0	14 ± 5
2	40.0	36 ± 5	30.0	34 ± 5	15.0	34 ± 5
3	60.0	50 ± 7	45.0	50 ± 7	17.0	36 ± 5
4	80.0	72 ± 4	60.0	84 ± 5	19.0	54 ± 7
5	100.0	98 ± 4	75.0	96 ± 4	21.0	72 ± 4
	10		**11**		**12**	
1	18.5	20 ± 7	9.0	22 ± 4	2.2	8 ± 4
2	27.75	36 ± 5	11.0	34 ± 5	2.75	14 ± 5
3	37.0	50 ± 7	13.0	50 ± 7	3.30	28 ± 4
4	46.25	66 ± 5	15.0	72 ± 4	3.85	36 ± 5
5	55.5	82 ± 8	17.0	94 ± 5	4.40	48 ± 4
	12a		**13**		**14**	
1	1.4	2 ± 4	13.0	8 ± 4	11.0	2 ± 4
2	1.75	8 ± 4	15.0	20 ± 7	13.0	8 ± 4
3	2.1	14 ± 5	17.0	34 ± 5	15.0	22 ± 4
4	2.45	28 ± 4	19.0	50 ± 7	17.0	36 ± 5
5	2.8	50 ± 7	21.0	66 ± 5	19.0	50 ± 7
	15		**16**		Permethrin	
1	10.0	8 ± 4	20.0	22 ± 4	0.0312	22 ± 4
2	20.0	24 ± 5	40.0	34 ± 5	0.0625	36 ± 5
3	30.0	36 ± 5	60.0	50 ± 7	0.1250	52 ± 8
4	40.0	44 ± 5	80.0	76 ± 5	0.1875	66 ± 5
5	50.0	56 ± 5	100.0	94 ± 5	0.2500	77 ± 4

[a]) Standard deviation (the value for both the positive and negative controls was 0 ± 0).

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