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ISOLATION AND CHARACTERIZATION OF AN ANTIMALARIAL AGENT OF THE NEEM TREE *AZADIRACHTA INDICA*[1]

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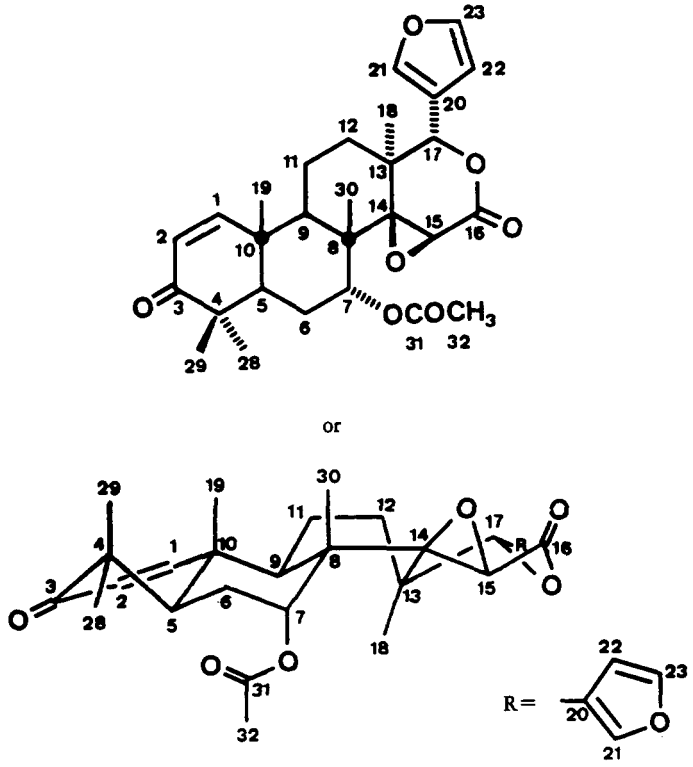
ABSTRACT.—The isolation and structure elucidation of gedunin [**1**], the antimalarial agent of *Azadirachta indica*, are reported. Its ^{1}H- and ^{13}C-nmr spectra were assigned by using one- and two-dimensional nmr spectroscopy, especially homonuclear and heteronuclear COSY, nOe difference, and COLOC experiments.

In a previous paper (1) we reported a bioassay procedure to test drugs against *Plasmodium falciparum*, the causative organism of malaria. By using this assay, we have found that the tetranortriterpenoid gedunin [**1**] is the active constituent of *Azadirachta indica* A. Juss. and *Melia azedarach* L. (Meliaceae). We report herein its isolation and the assignment of its ^{1}H- and ^{13}C-nmr spectra by one- and two-dimensional multipulse



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[1]This paper is dedicated to Prof. Dr. Dr. h.c. (H) Günther Snatzke on the occasion of his 60th birthday.

methods. These techniques have gained great importance since the early 1980s (2–10). The nmr experiments are presented by giving matrices containing all information extracted from these spectra.

We established molecular fragments from $^1H,^1H$ and $^1H,^{13}C$ connectivities ($^1H,^1H$ COSY and $^1H,^{13}C$ COSY, Figures 1 and 2). By this procedure, however, the fragments we obtained were terminated by either quarternary carbons or by oxygen atoms. To establish the full molecular structure, the fragments were combined by making use of long-range coupling evidence extracted from a 2D COLOC experiment (11) (Figure 2) and in part from an nOe difference experiment (Figure 3). The latter experiment allowed also the complete stereochemical assignment. The spectroscopic techniques are described in detail elsewhere (12).

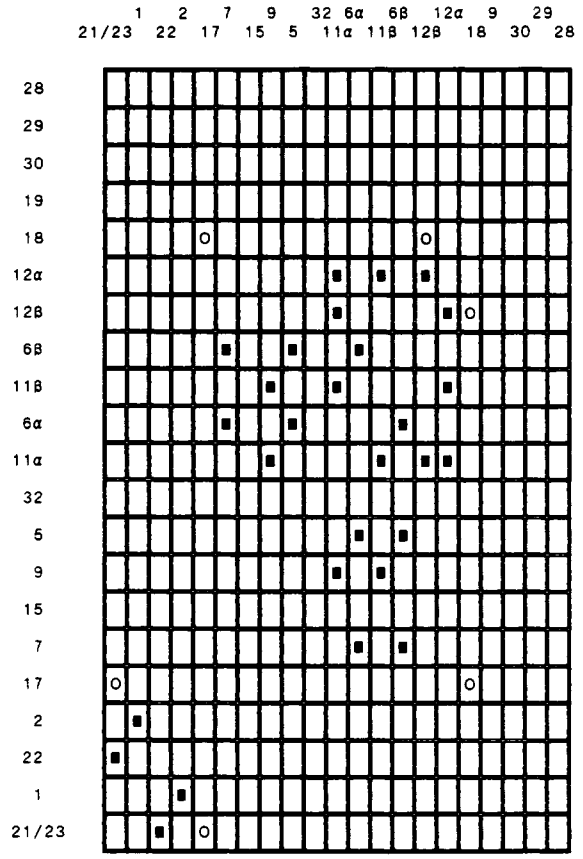

FIGURE 1. $^1H,^1H$ connectivity matrix. Squares represent significant cross peaks due to geminal and vicinal couplings, and open circles represent very weak cross peaks due to vicinal couplings with $J = 0$–1 Hz or long-range couplings.

Our results confirm with minor variations the ^{13}C-nmr reports published earlier for gedunin and its derivatives (13–16). A complete ^1H assignment, however, has not yet been presented in this class of compounds.

Rochanankij *et al.* (17) have recently reported the isolation of nimbolide from the leaves of *A. indica* var. *siamensis*, and they associated it with the antimalarial activity. Structurally, both nimbolide and gedunin are limonoid derivatives with a certain degree of chemical resemblance, most notably in the A-ring pattern. This is in line with our previous observation (1) regarding the integral role of ring A on the antimalarial activity of limonoids and the structurally related antimalarial quassinoids.

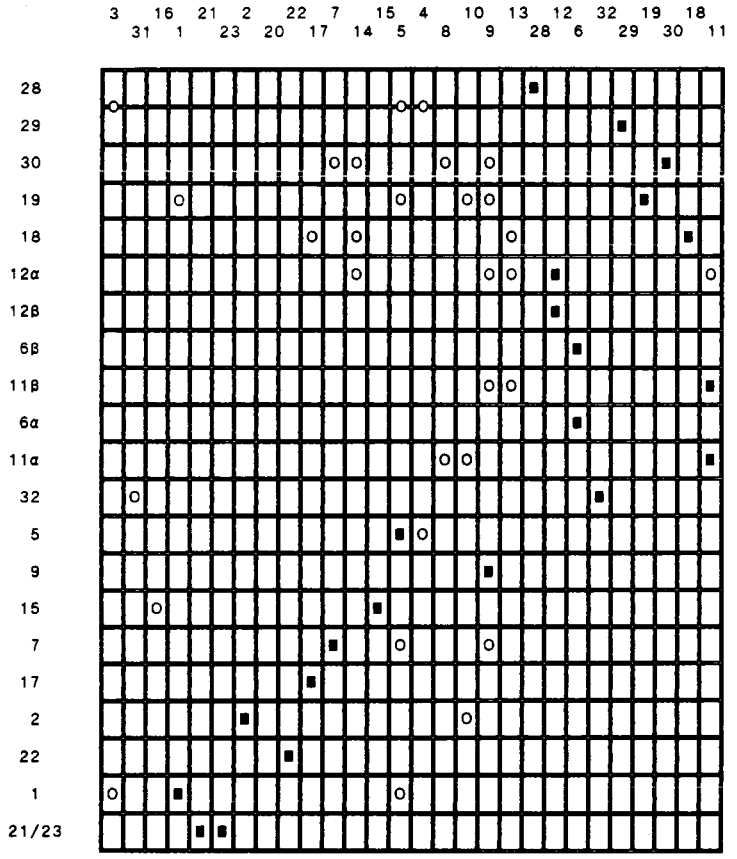

FIGURE 2. $^{1}H,^{13}C$ connectivity matrix. Squares represent one-bond couplings (from the HC COSY spectrum), and circles represent couplings via more than bond (from the COLOC spectrum). The position of the three circles between the two rows for a and b indicates that it cannot be decided which of them is the coupling partner of the respective carbon atom.

EXPERIMENTAL

PLANT MATERIAL.—Barks of *M. azedarach* and *A. indica* were collected in Khartoum, Sudan, and botanically authenticated by late Prof. H.M. Elamin. Herbarium specimens are deposited in the Department of Forestry, Soba-Khartoum.

EXTRACTION AND ISOLATION OF THE BIOACTIVE COMPOUND.—The ground bark of *M. azedarach* and *A. indica* (2 kg each) was percolated for 24 h with refluxing 70% MeOH. The MeOH was evaporated in a rotary evaporator and diluted with H_2O. The removal of lipophilic impurities was accomplished by repeated distribution between 2 liters of petroleum ether. The remaining aqueous extract was lyophilized. The lyophilizate was mixed with Si gel 60 (63–230 Merck) and chromatographed on Si gel column. The separation was monitored by tlc (Kieselgel 60, F_{245}, Merck) with petroleum ether/EtOAc.

Gedunin [**1**] was crystallized from MeOH, mp 220°, $[\alpha]^{20}D$ −44 ($CHCl_3$). Its high resolution mass spectrum gave a molecular ion corresponding to the formula $C_{28}H_{34}O_7$. The uv spectrum in MeOH exhibited maxima at 216 and 335 nm which are in line with data reported for the furan ring and the conjugated enone function. The ir spectrum revealed bands at 1668 (α,β-unsaturated carbonyl), 1709 (saturated ketone), and 1740 cm^{-1} (ester carbonyl). The bands at 875, 1502, and 3150 cm^{-1} are characteristic of a β-substituted furan ring. For ^{1}H and ^{13}C nmr see Table 1.

The antimalarial activity of gedunin was qualitatively assessed in vitro as described (1) using the microdilution technique essentially equal to that of Geary *et al.* (18). Gedunin had an IC_{50} of about 1μM after 48 h exposure (0.3 μM after 96 h), roughly equivalent to quinine.

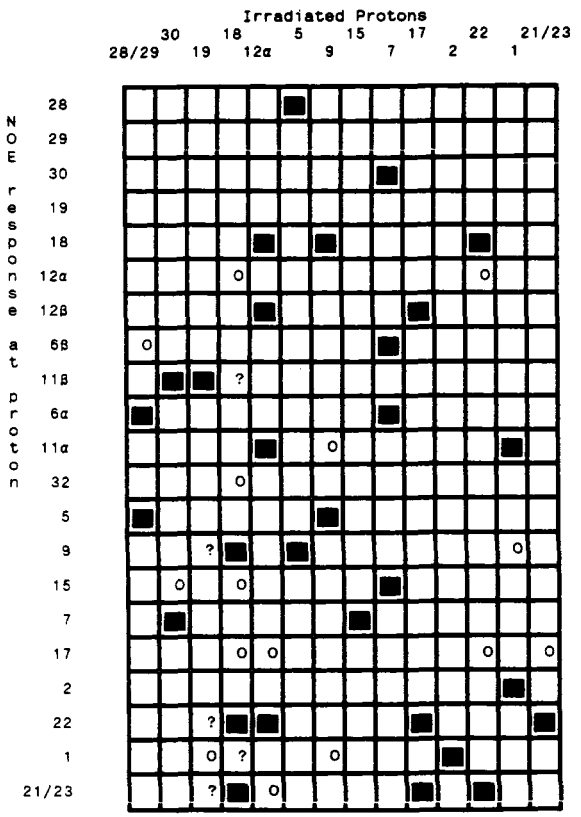

FIGURE 3. NOe matrix. Squares represent significant nOe-difference signals, open circles weak ones; "?" denotes signal responses which apparently are caused by an irradiation spill-over from protons with resonances near to the irradiation position.

TABLE 1. ^{13}C and ^{1}H Chemical Shifts and $^{1}H,^{1}H$ Coupling Constants of Gedunin [**1**] in $CDCl_3$.

Carbon		Proton	
C-1	157.0	H-1	7.07 (d, J = 10.2)
C-2	125.9	H-2	5.84 (d, J = 10.2)
C-3	204.0		
C-4	44.0		
C-5	46.0	H-5	2.12 (dd, J = 13.2, 2.3)
C-6	23.2	H-6α	1.92 (d, $J \approx 12$)
		H-6β	1.79 (t, $J \approx 12$)
C-7	73.2	H-7	4.52 (br s)
C-8	42.6		
C-9	39.5	H-9	2.46 (dd, J = 12.7, 6.2)
C-10	40.0		
C-11	14.9	H-11α	2.00 (m)
		H-11β	1.81 (m)
C-12	25.9	H-12α	1.56 (dd, J = 11–12)
		H-12β	1.70 (m)
C-13	38.7		
C-14	69.7		
C-15	56.8	H-15	3.50 (s)
C-16	167.4		

TABLE 1. Continued.

Carbon		Proton	
C-17	78.2	H-17	5.59 (s)
C-18	17.7	H-18	1.22 (s)
C-19	19.7	H-19	1.19 (s)
C-20	120.4		
C-21	143.1	H-21	7.39 (d, $J = 1.3$)
C-22	109.8	H-22	6.31 (dd, $J \approx 1.3$)
C-23	141.2	H-23	7.39 (d, $J = 1.3$)
C-28	27.1	H-28	1.03 (s)
C-29	21.2	H-29	1.04 (s)
C-30	18.3	H-30	1.12 (s)
C-31	169.9		
C-32	21.0	H-32	2.07 (s)

NMR MEASUREMENTS.—All spectra were recorded using a Bruker AM-400 spectrometer operating at 400.1 MHz (^{1}H) and 100.6 MHz (^{13}C). The solution was 1 mm in $CDCl_3$. The ^{1}H chemical shifts are referenced to $CHCl_3$ ($\delta = 7.24$) and the ^{13}C chemical shifts to the central peak of $CDCl_3$ ($\delta = 77.0$). Standard Bruker software was used.

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