HPLC gave an amorphous gum. This was identified as 8,30 epoxy swietenine acetate. ^{1}H NMR: δ7.39 (2H, *m*, H-21, 23), 6.3 (*m*, H-22), 5.37 (*s*, H-6), 4.97 (*s*, H-17), 4.77 (*d*, J = 10, H-3), 3.70 (3H, *s*, CO_2Me), 3.62 (*q*, J = 10, 2.6, H-2), 3.1 (*d*, J = 2.6, H-30), 2.07 (3H, *s*, Ac), 1.85 (6H, *m*, tiglate Me), 1.20, 1.02, 1.02, 0.92 (4 × C-Me); ^{13}C NMR: δ213.0*s*, 171.2*s*, 171.0*s*, 169.5*s*, 166.7*s*, 143.1*d*, 140.9*d*, 140.0*d*, 127.7*s*, 120.3*s*, 109.8*d*, 79.5*d*, 78.5*d*, 72.2*d*, 63.3*d*, 60.2*s*, 55.7*d*, 53.2*q*, 48.9*d*, 48.5*s*, 45.3*d*, 45.3*d*, 40.0*s*, 36.1*s*, 34.0*t*, 33.3*t*, 26.5*q*, 23.3*q*, 23.0*q*, 20.9*q*, 20.8*q*, 19.9*t*, 19.7*q*, 15.9*q*.

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ISOLATION AND STRUCTURE OF A QUASSINOID FROM *AILANTHUS GLANDULOSA**

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Key Word Index—*Ailanthus glandulosa*; Simarubaceae; 2-dihydroailanthone; quassinoid.

Abstract—A new quassinoid, 2-dihydroailanthone, has been isolated from the bark of *Ailanthus glandulosa*. Its structure was established on the basis of spectroscopic data and chemical evidence.

Previous studies on the constituents of *Ailanthus glandulosa* Desf. (syn. *Ailanthus altissima* Swingle) bark resulted in isolation of the bitter principles ailanthone **1a** ($C_{20}H_{24}O_7$) [1, 2]‖, amarolide ($C_{20}H_{28}O_6$) and acetylamarolide ($C_{22}H_{30}O_7$) [3, 4]. These compounds possess antiamoebic properties, although their rather high toxicity does not favour applications in therapy [5, 6]. On the basis of the reported biological activity and anticancer activities which are generally connected with the bitter principles isolated from the Simaroubaceae [7], we decided to further investigate the alcoholic extract of the plant bark. The search for minor constituents led to the isolation of a new member of the family, for which we have demonstrated the structure **1b**.

Elemental and mass spectral analyses (M^+ at m/z 378) indicated a $C_{20}H_{26}O_7$ molecular formula. By comparison with the previously described compounds from *Ailanthus glandulosa* these data were suggestive of a dihydroailanthone structure. Further confirmation for this structure was provided by IR [ν_{max} cm^{-1}: 3300–3500 (OH), 1715 (C=O)], ^{1}H NMR¶ (Table 1) and ^{13}C NMR spectra analyses (Table 2).

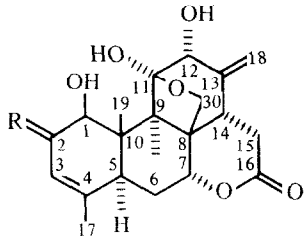
The lack of conjugation indicated by the UV spectrum (λ_{max} 205 nm) and the appearance of a signal corresponding to the extra oxymethine proton at δ4.59, by comparison to the ^{1}H NMR spectrum of **1a**, suggested the formulation of the structure **1b**. On this basis, the conclusion that the new compound was a 2-dihydroailanthone seems to be straightforward, and the stereochemistry of the new alcoholic function was demonstrated by the coupling constant between the protons on C-1 and C-2 (J = 8 Hz).

Unexpectedly, compound **1b** on treatment with acetic anhydride in pyridine gave a triacetate (**2**) [(mp 234–236°, EtOH); [M − 18]$^+$ at m/z 486], whose ^{1}H NMR spectrum clearly demonstrated that the hemiketal function was resistant to acetylation. In order to prove the proposed structure we decided to synthesize **1b** using ailanthone **1a** as starting material since the transform-

*Preliminary results were presented at the International Symposium on Natural Products, Tenerife, Spain, September 1980.

‖The stereochemistry at C-12 is here displayed according to our original proposal [1] and as recently confirmed by X-ray crystallography [10].

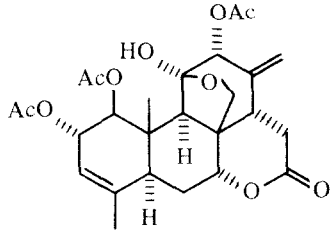
¶The 100 MHz proton spectrum was not satisfactorily resolved to enable us to draw a definite conclusion.



1a R = O

1b R = H, OH

1c R = OH, H



2

ation **1a** → **1b** requires only a stereoreductive process at C-2. In this context, it is important to stress that, while conventional reduction of the ketonic function in the quassinoids with sodium borohydride leads to products in which both the carbonyl group and δ-lactone unit are reduced [8], we found that sodium borohydride–sodium carbonate reduction, followed by filtration through an H^+ resin column, furnished alcohols in which the lactone group remained intact. Under these conditions, ailanthone (**1a**) underwent a reduction in a nearly stereospecific fashion, giving however the unwanted epimer **1c**, with only traces of the desired one being produced. Nevertheless, structure **1b** assigned to the title compound seems to be incontrovertible, as the analysis of its ^{1}H NMR (Table 1) and ^{13}C NMR spectra indicated (Table 2) [9].

While this manuscript was in preparation, a compound (shinjulactone A) was described, whose known characteristics are in accordance with those of the compound described here by us, but in which the stereochemistry at C-2 was not assessed [10].

EXPERIMENTAL

General. Mps were determined using a Kofler micro hot stage and are uncorr. IR spectra were determined in nujol. The MS were recorded on an AEI M.S. 902 spectrometer. UV spectra were measured in EtOH soln. ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 MHz and 200 MHz instruments using TMS as an internal standard. Merck's DC-Alufolien kieselgel 60 F_{254} and Merck's kieselgel 60 (70–130 mesh ASTM) were used for TLC and CC.

Extraction. Fresh bark (50 kg) of *Ailanthus glandulosa* was exhaustively extracted with EtOH at room temp. and the extract evaporated *in vacuo*. H_2O (2 l.) was added to the residue and the

Table 1. ^{1}H NMR spectra (400*, 200** and 100*** MHz) of compounds **1a**, **1b**, **1c** and **2**

Protons	1a*	1b*	1c**	2***
1-H	4.49 *s*	3.99 *d*	3.70 *d*	5.46 *m*
2-H	—	4.59 *m*	4.40 *m*	5.43 *m*
3-H	6.12 *q*	5.77 *m*	5.84 *m*	5.48 *m*
5-H	3.10 *dd*	2.66 *dd*	2.30 *d(br)*	—
6-H	2.03 *ddd*	1.92 *ddd*	2.01 *m*	—
6-H	2.24 *ddd*	2.02 *ddd*	1.82 *m*	—
7-H	4.66 *t*	4.56 *m*	4.50 *m*	4.42 *m*
9-H	3.55 *s*	3.27 *s*	3.10 *s*	2.48 *s*
12-H	4.58 *s*	4.57 *s*	4.45 *s*	5.24 *s*
14-H	2.92 *dd*	2.91 *dd*	2.75 *dd*	—
15-H$_\alpha$	3.70 *dd*	3.54 *dd*	3.54 *dd*	—
15-H$_\beta$	2.85 *dd*	2.78 *dd*	2.23 *dd*	—
17-H$_3$	1.78 *s(br)*	1.60 *s*	1.60 *s*	1.73 *s(br)*
18-Ha	5.19 *d*	5.18 *d*	5.10 *d*	5.24 *m*
18-Hb	5.28 *d*	5.26 *d*	5.18 *d*	5.28 *m*
19-H$_3$	1.54 *s*	1.66 *s*	1.38 *s*	1.39 *s*
30-Ha	3.61 *d*	3.65 *d*	3.52 *d*	3.50 *d*
30-Hb	4.12 *d*	4.15 *d*	4.02 *d*	3.82 *d*

J (Hz) **1a**: 3, 17 = 2; 5α, 6α = 3; 5α, 6β = 12; 6α, 7β = 3; 6α, 6β = 15; 6β, 7β = 3; 14β, 15β = 6; 14β, 15α = 13; 15α, 15β = 18; 17, 3 = 2; 18a, 18b = 2; 30a, 30b = 8. **1b**: 1α, 2β = 8; 3 $W_{1/2}$ = 9; 5α, 6α = 3; 5α, 6β = 13; 6α, 7β = 3; 6β, 7β = 3; 6α, 6β = 15; 7 $W_{1/2}$ = 4; 14β, 15β = 6; 14β, 15α = 13; 15α, 15β = 18; 18a, 18b = 2; 30a, 30b = 8. **1c**: 1α, 2α = 5; 5α, 6β = 12; 14β, 15β = 6; 14β, 15α = 13; 15α, 15β = 18; 18a, 18b = 2; 30a, 30b = 8. **2**: 30a, 30b = 8.

Table 2. ^{13}C NMR spectra (100.62* and 25.2** MHz) of compounds **1a**, **1b** and **1c**.

Carbons	1a*	1b*	1c**
C-1	84.4	83.9	79.0†
C-2	197.2	72.8	80.4†
C-3	126.1	127.0	124.2
C-4	162.5	134.9	135.3
C-5	45.0	45.0	45.1
C-6	26.3	26.2	26.7
C-7	78.6	79.2	79.4†
C-8	45.8	42.0	39.9
C-9	48.1	48.3	47.9
C-10	45.6	45.9	45.7
C-11	110.3	110.4	110.2
C-12	80.7	80.8	80.5†
C-13	147.4	148.0	148.5
C-14	42.6	42.1	41.1
C-15	35.4	35.5	35.3
C-16	169.3	169.4	169.4
C-17	22.4	21.1	21.6
C-18	118.3	118.1	117.8
C-19	10.2	10.6	11.4
C-30	72.3	72.7	72.5

†The assignments of these signals may be reversed.

mixture extracted with petrol (1 l. × 3) and then with $CHCl_3$ (1 l. × 3). The $CHCl_3$ soluble fraction (173 g) was chromatographed on a silica gel column. Elution with solvents of increasing polarity yielded **1a** [1], amarolide, acetylamarolide [2] and 100 mg of **1b**.

2-Dihydroailanthone (**1b**). White prisms, mp 262–263° (MeOH), IR ν_{max} cm^{-1}: 3500–3300, 1715, 1620 and 980. UV λ_{max} nm: 205. ^1H NMR and ^{13}C NMR (pyridine-d_5): see Tables 1 and 2. MS *m/z* 378 $[M]^+$, 266 and 250. (Found C, 63.18; H, 6.90. $C_{20}H_{26}O_7$ requires C, 63.48; H, 6.93%.)

Acetylation of **1b**. A soln of 2-dihydroailanthone (**1b**) (50 mg), pyridine (2 ml) and Ac_2O (0.5 ml) was kept at room temp. for 48 hr. Usual work up gave **2** (55 mg) which was crystallized from EtOH (Mp 234–236°). ^1H NMR ($CDCl_3$): see Table 1. MS *m/z* 486 $[M-18]^+$.

Sodium borohydride reduction of **1a**. A soln of 0.1 g of **1a**, 0.18 g of Na_2CO_3 and 0.03 g of $NaBH_4$ in 50 ml of H_2O was kept for 72 hr at 0°. The reaction mixture was filtered on Amberlite IR 120 (in H^+ form) column; the resin was then eluted with MeOH and the eluate evaporated. Chromatography of the residue on silica gel and elution with $CHCl_3$–MeOH (95:5) yielded 30 mg of semi-solid **1c**. ^1H NMR and ^{13}C NMR (pyridine-d_5): see Tables 1 and 2. UV λ_{max} nm: 205.

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NECATORIN, A HIGHLY MUTAGENIC COMPOUND FROM *LACTARIUS NECATOR*

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Key Word Index—*Lactarius necator*; Russulaceae; fungi; mutagenic compound; coumarin-cinnoline.

Abstract—The mutagen, necatorin, has been obtained from *Lactarius necator* and identified as 5-hydroxycoumaro (7,8-*c*)cinnoline.

Several species of mushrooms in the genus *Lactarius* have been found to contain mutagenic compounds in the Ames/*Salmonella* assay [1]. By far the highest mutagenic activity was found in *Lactarius necator* (Fr.) Karst. We have previously reported [2] the isolation of a mutagenic compound in crystalline form by HPLC of an extract of *Lactarius necator*. In the present communication we report its tentative identification.

Interpretation of the mass spectrum reveals the compound to be a coumarin on the basis of two sequential losses of carbonyl ($[M-CO]^+$ is the base peak) [3]. The coumarin structure is further confirmed by the photosensitizing effect of the compound; this effect is similar to that of several furanocoumarins [4]. This structure is further confirmed by two doublets in the ^1H NMR spectrum at δ6.55 and δ7.55 ($J = 9$ Hz) assigned to protons three and four in a coumarin system. The benzo(c)cinnoline structure is revealed by the loss of nitrogen after two losses of carbonyl in the mass spectrum. The benzo(c)cinnoline structure is in accordance with the presence of a four proton system in the ^1H NMR spectrum. The third loss of carbonyl is indicative of the hydroxyl group, which is confirmed by proton exchange in the ^1H NMR spectrum in CF_3COOD. The presence of a