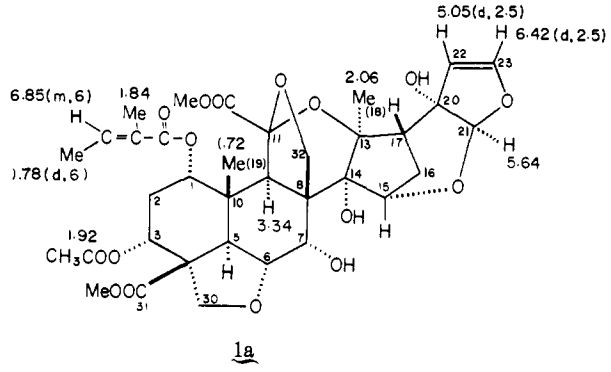
Structure of the Insect Phagorepellent Azadirachtin. Application of PRFT/CWD Carbon-13 Nuclear Magnetic Resonance

Sir:

Preliminary studies[1] using the leaf extracts of *Azadirachta indica* (Indian neem tree) collected in Mombasa, Kenya, indicated the presence of compound(s) which induced pronounced morphological changes in *Antestiopsis* (coffee bug) upon topical application. Previous studies on this and the closely related species *Melia azedarach* had disclosed the presence of two active compounds, i.e., meliantriol[2] (locust antifeedant) and azadirachtin[3] (very active insect phagorepellent[4] and systemic growth disruptor[5]), as well as other liminoids[6] and triterpenoids.[2,7] We propose structure **1** for azadirachtin,[8] which was encountered during attempts to isolate the morphologically active principles mentioned above.

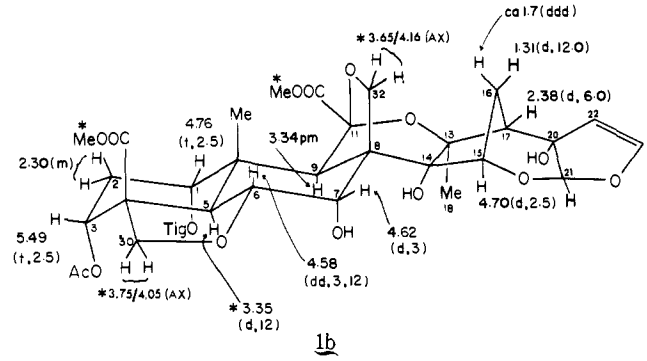
In 1972 Morgan and coworkers[3c] undertook detailed structural studies of azadirachtin, $C_{35}H_{44}O_{16}$, and proposed the presence of partial structures **2a/2b,** a tiglate moiety, and other functional groups. A gross *C*-seco-triterpenoid nature was assigned[3c] on the basis of selenium dehydrogenation of the lithium aluminum hydride reduction products, which yielded di-, tri-, and possibly tetramethylnaphthalenes (GC, MS) but no phenanthrenes.

Extraction of 300 g of seeds[9] with ethanol followed by silica gel chromatography (3% acetone–ether, and then a 1:1 mixture of chloroform–ethyl acetate) and preparative TLC



1a

^{1}H NMR data ($CDCl_3$) are shown in ppm (multiplicity, *J* values). The COOMe groups have ^{1}H NMR signals at 3.65 and 3.76 ppm. See **1b** for ^{1}H NMR data not depicted here.



1b

^{1}H NMR peaks with * including two COOMe at 3.65 and 3.76 ppm are close to 3.91 ppm; carbons attached to these protons appear as singlets in ^{13}C NMR upon irradiation at 3.91 ppm (see asterisked peaks in Figure 1a).

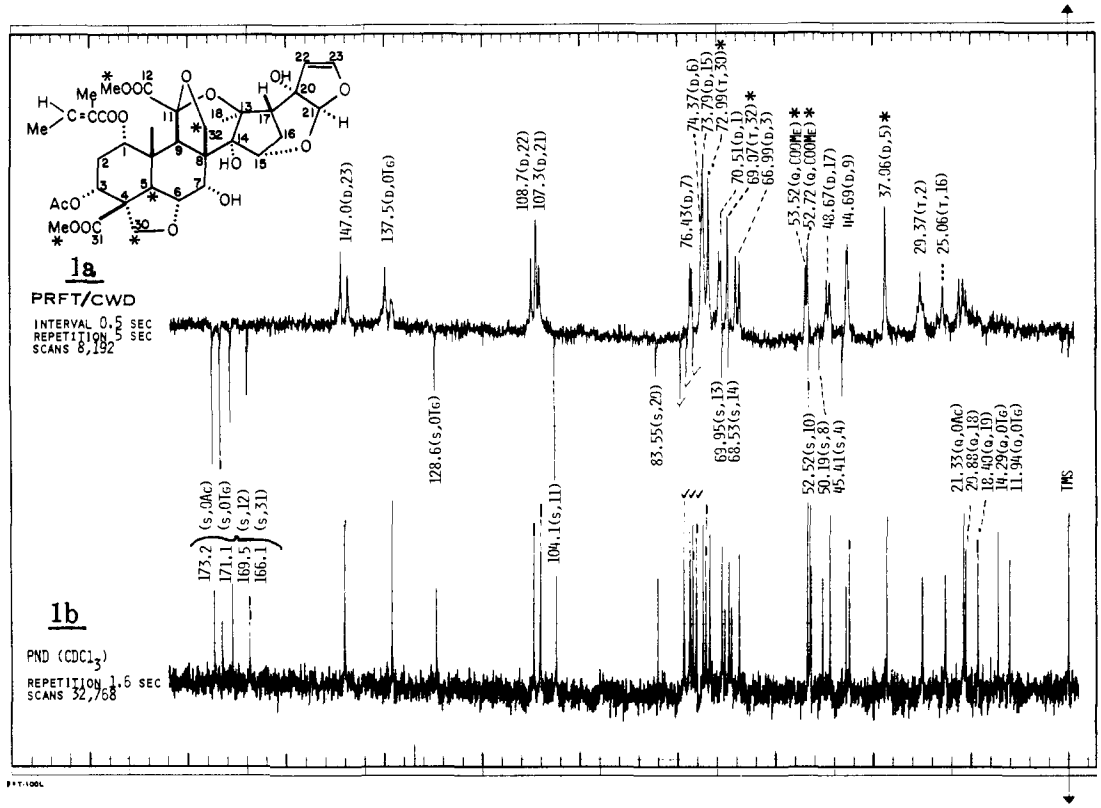
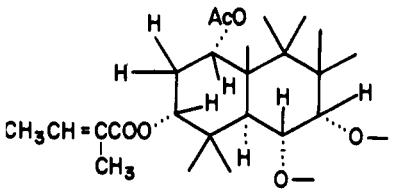
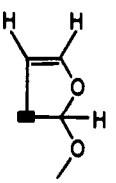


Figure 1. (a) PRFT/CWD (narrow band irr at 3.91 ppm) spectrum of **1** in $CDCl_3$; interval time, 0.5 sec; repetition time, 5 sec; scans, 8192. Carbons (with *) attached to protons absorbing close to 3.9 ppm (see structure **1b** for chemical shifts) appear as peaks with very small or nil residual couplings; these have not been taken into account in the multiplicities shown here. Numerals in parentheses denote carbon position. The C-30 and C-32 peaks assignments could be reversed. (b) PND spectrum of **1** in $CDCl_3$; repetition time, 1.6 sec; scans, 32,768. A JEOLCO PS-100 instrument was used. Peaks with check marks denote $CDCl_3$ signals.



2a



2b

yielded 800 mg of pure but amorphous azadirachtin.[10] Structural studies including *simultaneous* usage of partially relaxed Fourier transform (PRFT)[11] and continuous wave decoupling (CWD) ^{13}C NMR techniques carried out on azadirachtin has led to the full expression **1**.

Azadirachtin (uv (MeOH) 217 nm (ϵ 9100); CD (MeOH), no extrema above 220 nm; ir (KBr) 3410 cm^{-1} (OH), 1745, 1720, 1700 (esters), 1645, 1620, 843 (double bonds); ir (chf. concentration variation) 3580 cm^{-1} (free OH), 3465 (intramolecular H bonded OH), 3380 (intermolecular H bonded OH)) contains the following groups (see **1a**, and Figure 1): one acetate,[12] tiglate, two methoxycarbonyls, two olefinic protons in addition to that in the tiglate, and two quaternary methyls. In agreement with Morgan,[3c] it also contains one secondary and two tertiary hydroxyls (^1H NMR in DMSO-d_6) and part structure **2b**.

The nature of the 35 carbons was clarified by a combination of ^{13}C NMR techniques, i.e., proton-noise decoupling (PND, Figure 1), CWD, PRFT, and combined PRFT/CWD. The PRFT/CWD technique greatly simplifies assignments of congested ^{13}C NMR peaks since in addition to spectrum simplification by partial relaxation (i.e., separation into negative and positive peaks), one can also obtain peak multiplicities. For example, in the PRFT spectrum shown in Figure 1a, where a simultaneous CWD frequency (narrow band) at 3.91 ppm was applied, peaks due to the four carbonyl and eight quaternary carbons, including the C-11 ketal at 104.1 ppm, appear as negative singlets, and thereby make multiplicity detection of the positive peaks more facile. The asterisked peaks are due to carbons attached to protons absorbing in the range centered around 3.9 ppm and hence appear as singlets. The simplifications achieved in the 44–54 and 66–77 ppm regions should be noted. In the example shown, the high-field methyl peaks are nulled but these can be readily identified in the PND spectrum (Figure 1b). Similar detailed ^{13}C NMR studies were carried out on the closely related *C*-seco-tetranor-triterpenes salannin[6b] and nimbin[6a] in order to corroborate these assignments.

The following five isolated proton systems (see **1b**) were disclosed in addition to those depicted in **1a** by extensive usage of solvent effects, e.g., addition of C_6D_6 to $CDCl_3$, addition of Eu(fod)$_3$, and consideration of ^{13}C NMR patterns (Figure 1): (i) AX pattern (J = 10 Hz) at 3.75 and 4.05 ppm (30-H_2); (ii) AX pattern (J = 9.5 Hz) at 3.65 and 4.16 ppm (32-H_2); (iii) 1-H(eq)/2-H_2/3-H(eq); (iv) 5-H(ax)/6-H(ax)/7-H(eq); and (v) 15-H/16-H_2/17-H. Systems i, iii, and iv are similar to those encountered in salannin,[6b] whereas systems ii and v are unique for azadirachtin. There is also a one-proton singlet at 3.34 ppm (9-H).

Acetylation with neat acetic anhydride by refluxing under nitrogen for 10 min gave the tertiary acetate at C-14 as the sole acetylation product; this is presumably formed by acyl migration from C-7, the further C-7 acetylation being blocked by steric hindrance. The ^{13}C NMR peaks which underwent significant shifts are listed in Table I. These carbons, *together with their proton systems*, if any, should be placed in close proximity, i.e., α or β, to the tertiary acetoxyl carbon (C-14). The variations in the direction of shifts are probably due to different conformations of the six-membered ring comprising the C-11–C-13 ether bridge as well as the loss of H bonding of 14-OH upon acetylation.

Table I. Chemical Shift Differences in the ^{13}C NMR of Azadirachtin and Its 14-Acetate

	C-14	C-8	C-13	C-15	C-9	C-32	C-18
14-OH	68.53	50.19	69.95	73.79	44.69	69.07	18.40
14-OAc	64.77	49.25	68.61	74.39	48.00	70.11	18.55
Δ ppm	3.76	0.94	1.34	−0.60	−3.31	−0.94	−0.15

An unexpected observation was the shift of the 4.76 ppm ^1H NMR signal in azadirachtin (attached to carbon bearing the tiglate group[3c]) to 5.17 ppm in the 14-acetate. However, the corresponding ^{13}C NMR signal remained constant at 70.51 ppm in both compounds, and therefore the ^1H NMR shift must be due to an anisotropic effect, caused by different orientations of the tiglate group or the 11-COOMe group. Since the tiglate bearing methine ^1H NMR signal[3c] is affected by 14-acetylation, it should be located at C-1 (as in salannin[6b]) and not at C-3.

Configuration of the dihydrofuran should be β (see **1b**) with the 20-OH cis to 13-Me in view of the low-field methyl shift (2.06 ppm);[12] if it were α, the 13-Me signal would be at a higher field due to shielding by the 22-ene. A long-range coupling was observed between the 1.72 ppm Me and 9-H thus showing their trans-periplanar relation as well as locating this Me group at C-10. Irradiation of the 2.06 ppm Me signal (13α) resulted in a 6.4% NOE at the 9α-H 3.34 ppm signal. The structure thus derived is biogenetically related to salannin,[6b] nimbin,[6a] and nimbolin B.[6d] The neem tree seeds gave in addition to azadirachtin, 550 mg of salannin.

Acknowledgments. We are grateful to Professor K. Overton, Glasgow, for a generous supply of nimbin and salannin.[13]

References and Notes

(1) Carried out in collaboration with K. Leuschner, Kiambu Research Station, Coffee Research Foundation, Kenya.
(2) D. Lavie, M. K. Jain, and S. R. Shpan-Gabrielith, *Chem. Commun.*, 910 (1967).
(3) (a) J. H. Butterworth and E. D. Morgan, *Chem. Commun.*, 23 (1968); (b) *J. Insect Physiol.*, **17**, 969 (1971); (c) J. H. Butterworth, E. D. Morgan, and G. R. Percy, *J. Chem. Soc., Perkin Trans. 1*, 2445 (1972); (d) E. D. Morgan and M. D. Thornton, *Phytochemistry*, **12**, 391 (1973).
(4) When tested against *Schistocerca gregaria* (desert locust) the limiting concentration to cause 100% inhibition of feeding is 40 μg/l., or when impregnated onto filter paper 1 ng/cm^2: ref 3b, d.
(5) J. S. Gill and C. T. Lewis, *Nature (London)*, **232**, 402 (1971); C. N. E. Ruscoe, *ibid.*, **236**, 159 (1972).
(6) (a) M. Harris, R. Henderson, R. McCrindle, K. H. Overton, and D. W. Turner, *Tetrahedron*, **24**, 1517 (1968); (b) R. Henderson, R. McCrindle, A. Melera, and K. H. Overton, *ibid.*, **29**, 1525 (1968); (c) J. D. Connolly, K. L. Handa, and R. Mc Crindle, *Tetrahedron Lett.*, 437 (1968); (d) D. E. U. Ekong, C. O. Fakunle, A. K. Fasina, and J. I. Okogun, *Chem. Commun.*, 1166 (1969); (e) C. R. Mitra, H. S. Garg, and G. N. Pandey, *Tetrahedron Lett.*, 2761 (1970); (f) D. Lavie, E. C. Levy, and M. K. Jain, *Tetrahedron*, **27**, 3927 (1971).
(7) C. K. Chiang and R. C. Chang, *Tetrahedron*, **29**, 1911 (1973).
(8) Bioassays (feeding and injection) carried out with the following insects have shown that azadirachtin is a potent antifeedant: *Heliothis virescens* (tobacco bud worm) larvae, *Pieris brassica* (cabbage moth) adults, *galleria mellonella* larvae, and *Teticuli termis* (termite larvae). These bioassays were kindly performed by Drs. G. Ludwick and G. Staal, Zoecon Corporation (H. virescens), and Professor H. Röller, Texas A&M University (other insects).
(9) We are grateful to Drs. W. Hauserman and W. F. Wood, International Centre of Insect Physiology and Ecology, for collections. Yield of azadirachtin is small from whole fruits.
(10) Azadirachtin is unstable in solution, and a 30% decomposition was noticed when kept in a ^{13}C NMR tube, $CDCl_3$ solution, for 3 days.
(11) R. L. Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.*, **48**, 3831 (1968); R. Freeman and H. D. W. Hill, *Ibid.*, **53**, 4103 (1970); A. Allerhand, D. Doddrell, V. Gushko, D. W. Cochran, E. Wenkert, P. J. Lawson, and F. R. N. Gurd, *J. Am. Chem. Soc.*, **93**, 544 (1971); D Doddrell and A. Allerhand, *Proc. Nat. Acad. Sci. U.S.*, **66**, 1083 (1971); G. C. Levy, J. D. Cargioli, and F. A. L. Anet, *J. Am. Chem. Soc.*, **95**, 1527 (1973); F. W. Wehrli, *J. Chem. Soc. C*, 379 (1973); K. Nakanishi, V. P.

Gullo, I. Miura, T. Govindachari, and N. Visa-Wanathan, *J. Am. Chem. Soc.*, **95**, 6473 (1973); K. Nakanishi, R. Crouch, I. Miura, X. Dominguez, A. Zamudio, and R. Villarreal, *ibid.*, **96**, 609 (1974).
(12) One of the two "acetates" proposed by Morgan[3] was found by ^{13}C NMR to be an exceptionally low-field quaternary methyl (^1H NMR 2.06 ppm).
(13) Supported by NIH AI-10187.

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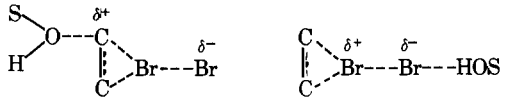
Role of the Solvent in Bromine Additions to Olefins. Solvent Independence of the Charge Distribution in Transition States and Intermediates

Sir:

Solvent dependence of stereoselectivity in aryl olefin bromination[1] has been attributed to competition between bromine bridging and *nucleophilic* solvation in the carbonium ion intermediate **1**: the more the positive charge is solvated, the less the importance of bromine assistance. Specific solvation would favor the carbonium over the bromonium



1

2

ion intermediate and would lead to low stereoselectivity. However, solvation of the 1-pentene bromination transition state **2**, known to be bromonium ion-like,[2] is essentially *electrophilic*.[3] Insofar as the intermediate closely resembles the transition state,[3] these observations appear contradictory. The contradiction would be resolved if the solvation of carbonium ion intermediate[4] were substantially different from that of bridged bromonium ions. We now report results on the bromination of styrene (I) and *trans*-stilbene (II), which negate this hypothesis.

Rate constants of the molecular bromine addition to olefins I and II in solvents whose Y[5] varies over 5 units are shown in Table I and compared with those of 1-pentene.

Solvent effects on 1-pentene, styrene, and stilbene bromination are remarkably similar.

$$\log k_{sty} = 0.83 \log k_{1\text{-pe}} - 1.73 \quad (R = 0.997,\ s = 0.004) \quad (1)$$

$$\log k_{stil} = 1.02 \log k_{1\text{-pe}} + 0.91 \quad (R = 0.999,\ s = 0.005) \quad (2)$$

For 1-pentene, Winstein's equation[5] in its simplest form applies[3]

$$\log (k/k_0) = 1.16Y \quad (3)$$

From eq 1-3, m values of 0.96 and 1.20 were obtained for styrene and stilbene, respectively. Solvent effects on 1-bromoadamantane solvolysis, where nucleophilic solvation is impossible, are correlated[6] by Y with $m = 1.20$. This value corresponds to a transition state where the leaving group is a bromide ion, as in bromination. We can, therefore, conclude that nucleophilic solvation is insignificant in bromination of 1-pentene and stilbene which have similar m values (1.16 and 1.20, respectively). Although the m value for styrene is smaller (0.96), this decrease cannot be attributed to nucleophilic solvent assistance since this generally leads to much smaller values,[6] e.g., for isopropyl bromide solvolysis, $m = 0.43$.

Table I. Solvent Effects on the Bromination of Styrene, *trans*-Stilbene, and 1-Pentene

Solvent	Y[b]	k_{Br_2}[a] l. mol^{-1} sec^{-1} Stilbene	Styrene	1-Pentene
H_2O	3.4	$7.8\ 10^5$ [d]	$1.1\ 10^7$ [g]	$2.5\ 10^7$ [h]
M-50[c]	1.97		$2.3\ 10^6$	$9.15\ 10^5$ [h]
CF_3CH_2OH	1.04	$1.7\ 10^3$		$6.85\ 10^4$ [h]
CH_3OH	−1.09	$1.1\ 10$ [e]	$1.16\ 10^3$ [g]	$3.80\ 10^2$ [h]
CH_3COOH	−1.64	$1.8\ 10^{-2}$ [f]	8.4[f]	1.13

[a] Rate constants are measured at 25° by electrometric and spectrophotometric methods described previously, ref 2, 3, and 17. [b] Reference 5 and 6. [c] 50% (volume) aqueous methanol. [d] Reference 16. [e] Reference 17. [f] Reference 19. [g] Reference 18. [h] Reference 3.

Thus no nucleophilic assistance can be detected regardless of whether the transition state is carbonium or bromonium ion-like. Nevertheless, the solvent operates in two ways: firstly, through a medium effect on the magnitude of the charge separation between cationic and anionic parts of the transition state and secondly by electrophilic solvation demonstrated, at least for methanol, by the solvent isotope effect,[3] $k_H/k_D = 1.40$. Since specific solvation of the cationic part does not exist, the solvent cannot influence significantly the distribution of the positive charge between the olefinic carbon atoms and the bromine.

On the other hand, we have shown elsewhere[4] that the charge distribution depends on substituent character. There is, therefore, a distinct separation between the factors which determine charge magnitude (solvent) and charge distribution (substituents). This view agrees with Schleyer's observations[7] in the β-aryl ethyl ester solvolysis where the importance of neighboring phenyl assistance depends not on the solvent but only on the ring substituents.

Consistent with the concept of a transition state with only the anionic part solvated is the fact that substituent effects on the bromination of alkenes are closely similar, regardless of the solvent.[8] However, recently,[9] Olah et al. claim that substituent effects are enhanced on going from Freon 113 at −35° to methanol at 25°.

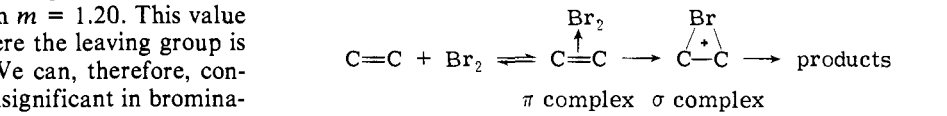
$$\log k\ (\text{Freon 113, } -35°) = 0.69 \log k\ (\text{MeOH, } 25°) + \text{Cte} \quad (R = 0.969)$$

This result is surprising since we have shown[3] that structural effects are very similar in methanol and Freon 112.

$$\log k\ (\text{Freon 112, } 25°) = 1.10 \log k\ (\text{MeOH, } 25°) - 5.99 \quad (R = 0.965)$$

$$\log k\ (\text{Freon 112, } 25°) = 1.45 \log k\ (\text{Freon 113, } -35°) + \text{Cte} \quad (R = 0.956)$$

Now, $\rho = f(1/T)$; i.e., substituent effects should decrease with rise in temperature, and it is inconceivable that the change in solvent from Freon 113 to Freon 112[10] should modify the mechanism enough to invert the normal trend of ρ with temperature. According to Olah's interpretation, the transition state would be less charged, further from the σ



π complex σ complex