The Structure of Harrisonin

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In the following we forward structure 1 for harrisonin isolated from the root bark of an East African shrub. Harrisonin has insect antifeeding, cytotoxic and antibacterial properties.

The East African shrub Harrisonia abyssinica Oliv (Simarubaceae)("Msabubini" or "Mpapura-doko" in Swahili) is widely used in various folk remedies, including treatment for bubonic plague, hemorroid, snake-bite, etc.[1] The chopped root bark (650g) of the shrub collected near Mombasa, Kenya gave after work-up ca. 70 mg of "harrisonin" 1 and 25 mg of obacunone 2[2], both in crystalline form. Spectral studies of harrisonin led to structure 1 which contains an unusual α-hydroxy-α'-hemiketal ketone moiety; it is not clear whether this grouping is present in nature or is derived from a hydroxy-α-diketone during isolation. As far as we know it is the only known derivative of obacunone.

The current studies were initiated because the crude aqueous methanol extract exhibited antifeedant properties[3] against the common East African crop pest, the monophagous Spodoptera exempta (army worm). The crude extract was also active against gram-positive microorganisms.[4] The isolation of the bioactive

principle(s) was followed by antifeedant activity and antibacterial tests. The antifeedant tests were carried out by dipping 2 cm leaf disks into acetone solutions of the extracted fractions for two seconds. Both tests led to the same compound, harrisonin 1, the final activity being 20 ppm for antifeeding (quite potent) and 5 μg/ml against Bacillus subtilis.[4] In addition it was found that harrisonin exhibits cytotoxicity at the level of 2.2 μg/ml (KB test).[5]

The nature of all 27 carbon atoms in harrisonin (see 1a) were clarified by proton-noise decoupled (PND), undecoupled, and partially relaxed Fourier transform (PRFT)[6] spectra, shown with their chemical shifts in Fig.1. As is generally the case for terpenoids, the T_1 relaxation time for various carbons increase in the sequence of methylene, methine, methyl and carbons which bear no hydrogens.[7] This is the case shown in the PRFT spectrum, where the negative peaks below 25 ppm consist only of carbonyl, quaternary carbons and the methoxyl carbon, whereas the positive peaks are all due to carbons bearing one hydrogen. In the high-field region the two conspicuous positive peaks are assignable to methylene carbons, which appear as triplets in the undecoupled spectrum (although they are not specificially designated in Fig.1 due to spectral congestion). The methyl peaks (discussed below) appear either as negative or positive peaks under the conditions of the PRFT measurement.

Several long-range ^{13}C-H couplings are observed in the undecoupled spectrum. Of particular significance was that of the two lactonic carbons; the 167.9 ppm peak was a simple doublet,

FIG. 1

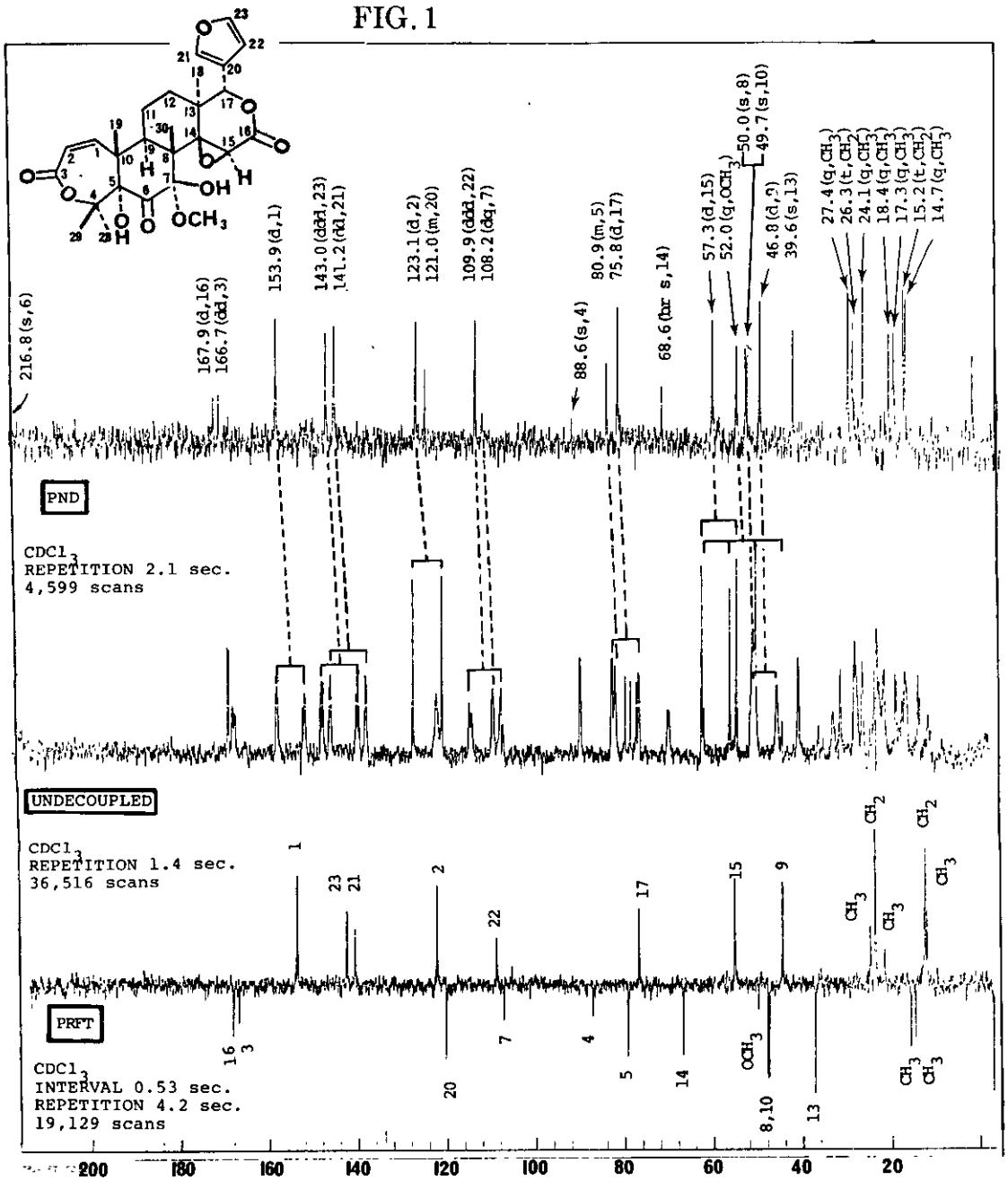


Fig.1 Cmr spectra of harrisonin, 25 MHz, $CDCl_3$

whereas the 166.7 ppm peak was a doublet of doublets (dd) arising from further coupling to a second proton, 2-H, in addition to 1-H. The 108.2 ppm signal appears in a range typical for ketalic carbons; the unusual doublet of quartets (Fig.1 undecoupled run) was converted into a quartet upon addition of D_2O and hence this indicated the presence of a hemiketal function (at C-7). Other long-range ^{13}C-H couplings are seen in the typical furanoid carbons C-21,22 and 23.

The low chemical shift of the 153.9 ppm doublet suggested that an α,β-unsaturated carbonyl function was present. However the uv, ir and cd spectra all showed the absence of enones, and hence this was assigned to an α,β-unsaturated lactone; the absence of a uv band in the 220 nm region typical for unsaturated lactoned was tentatively attributed to a twisted lactone.

The region 90-55 ppm falls into that of oxygen bearing carbons. Of the five peaks here, two are methine groups (see PRFT and undecoupled spectra) and furthermore the high chemical shift of one of the methine peaks at 57.3 ppm is consistent with a CH group of an epoxide.

Pertinent points in the pmr spectrum (Fig.2) were the following: a) There are two intramolecularly bonded tert-hydroxyl groups as indicated by the sharpness and the low chemical shifts of the 5.05 and 3.67 ppm peaks; b) the 5.70 ppm singlet was weakly coupled to 21-H and this together with its low chemical shift leads to the moiety O-C<u>H</u>-furan (β-substituted); c) the ill defined "triplet" at 3.00 ppm suggested that a C<u>H</u>-CH_2-CH_2 group was present and that virtual coupling may be involved; d) the two

FIG. 2

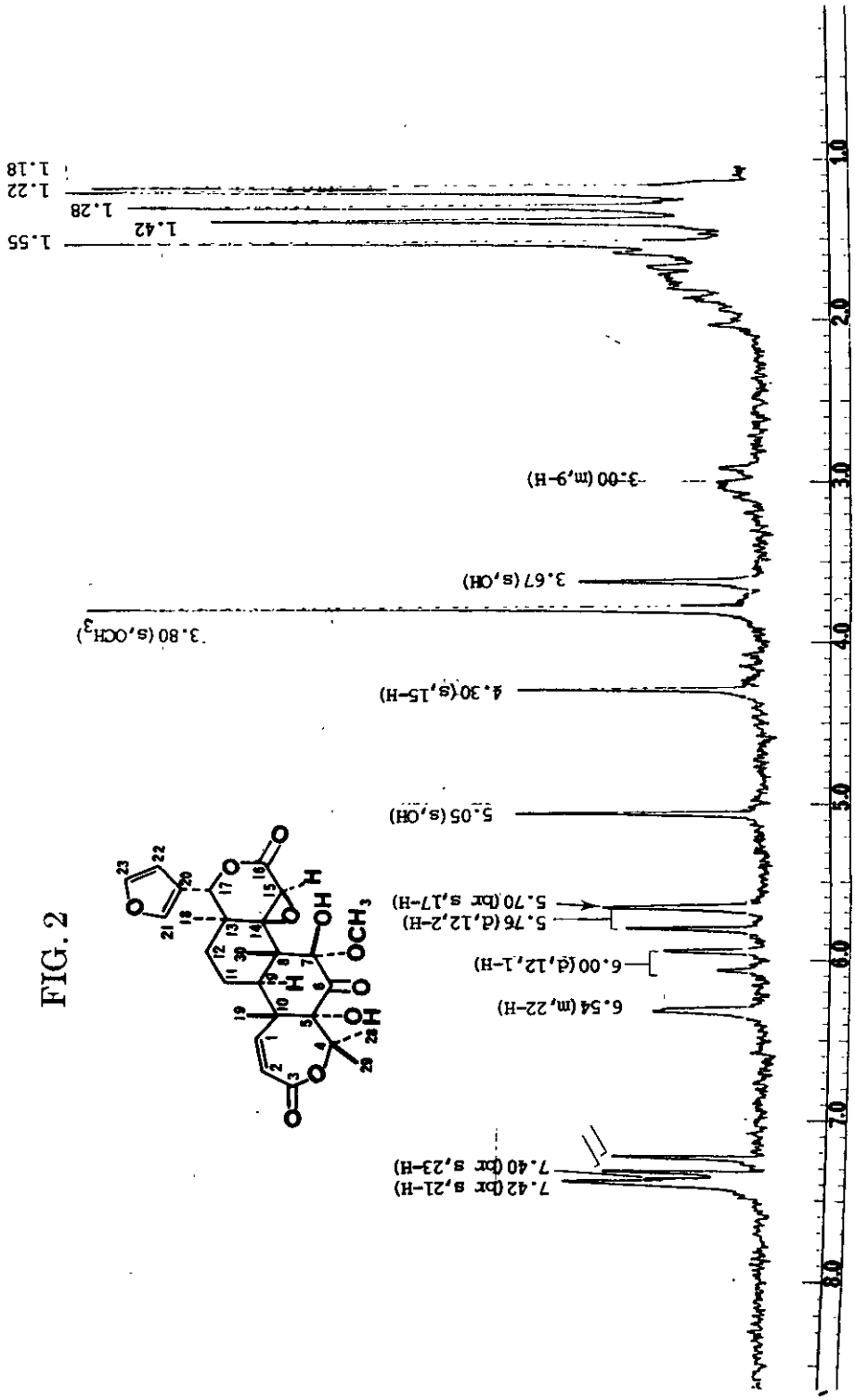


Fig.2 Pmr spectrum of harrisonin, 100 MHz, $CDCl_3$

✓ = C_6H_6, $CHCl_3$

olefinic peaks at 6.00 and 5.76 ppm constitute an AB quartet, the unusually high chemical shifts again suggesting that the lactone is twisted.

Cmr and pmr data as well as other spectral data of the second compound characterized it as being obacunone[2] (see structures 1b, 2b and Fig.3). The β-substituted furanoid pmr peaks due to 21-H and 23-H and the 9-H signal were clarified by the 220 MHz spectrum (Fig.3 inset). Similarities in the spectral data for compounds 1 and 2 clearly showed that the gross structures were the same.

The pmr spectrum of harrisonin 1 (Fig.2) differs from that of obacunone 2 (Fig.3) in that the former lacks the 5-H/6α-H/6β-H three proton system in the region 3.00-2.00 ppm, and that the 15α-H 4.30 ppm peak in 1b is down-shifted in comparison to the 3.64 ppm peak in 2b. The difference in cmr shifts of the C-4 and C-5 peaks between 1a and 2a showed that one of the two tert-hydroxyl groups (pmr data, vide supra) in harrisonin was attached to C-5. Cmr data had shown that 1 contained a hemiketal function. This was placed at C-7 rather than at C-6 in view of the large difference in the 15α-H signals (see 1b and 2b). The low shift of the 3.00 ppm 9-H signal in 1b, as compared to the 2.10 ppm value in 2b, can now be accounted for by the presence of two 1,3-diaxial oxygen functionalities in 1b. The remaining ketone function of 1 (1709 cm^{-1} ir band in $CHCl_3$) should then be placed at C-6.

The difference in the cd spectra of 1 and 2 (Fig.4) is consistent with their structures although that of 1 is anamolous.

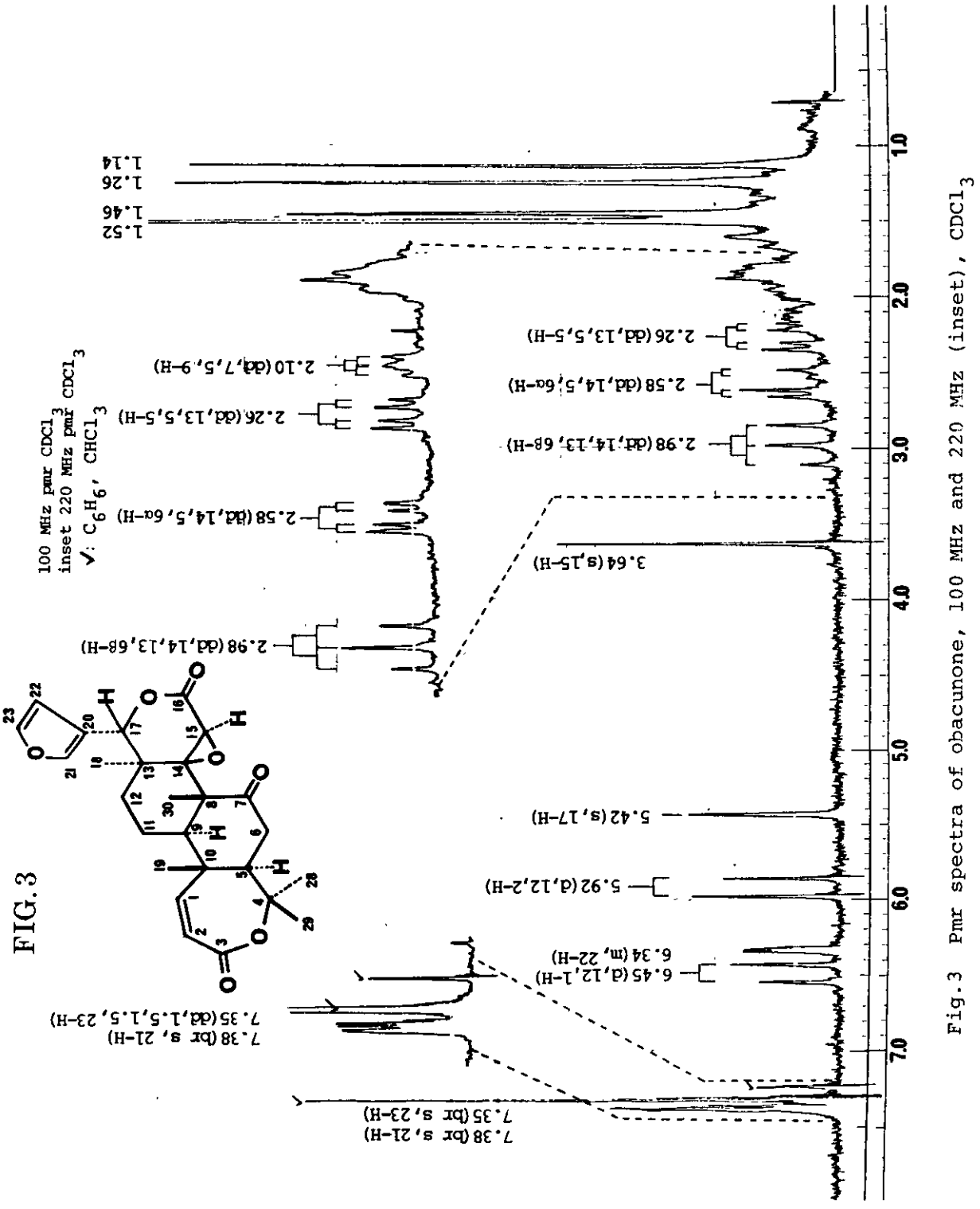
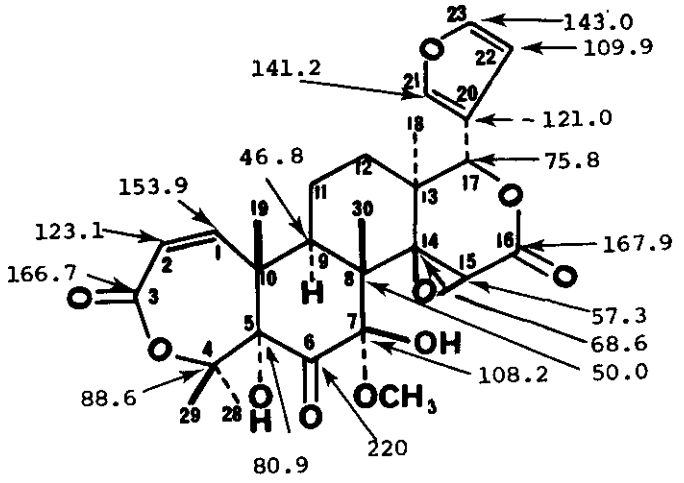


FIG. 3

Fig. 3 Pmr spectra of obacunone, 100 MHz and 220 MHz (inset), $CDCl_3$



Harrisonin

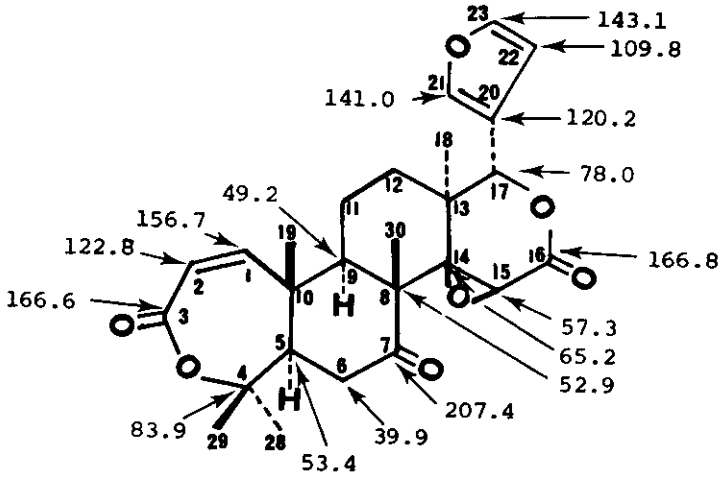
1a:cmr data in $CDCl_3$

$C_{27}H_{32}O_{10}$, m.p. 155-156°

CI(isobutane)/MS: 517(MH^+)

uv(CH_3OH): end

ir($CHCl_3$): 3490(intra. H-bond), 1760(sh)
1741, 1709, 1627(C=C), 875(furan)



Obacunone

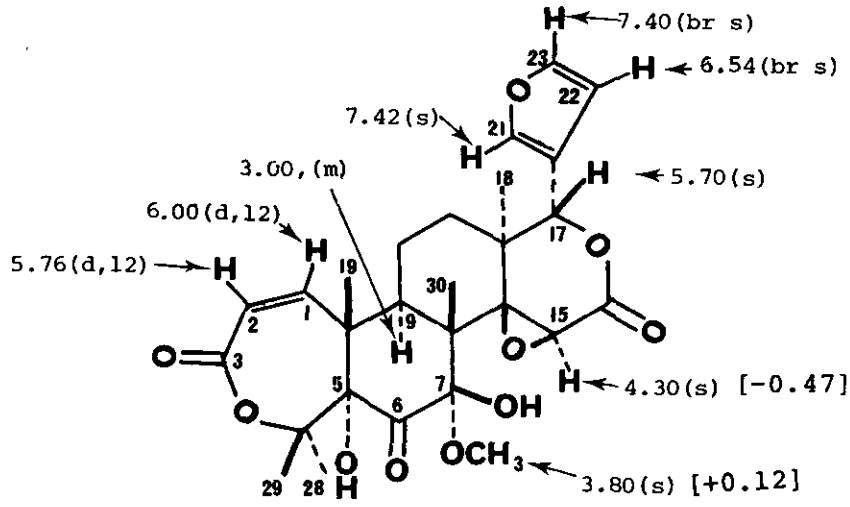
2a:cmr data in $CDCl_3$

$C_{26}H_{30}O_7$, m.p. 226-228°

CI(isobutane)/MS: 455(MH^+)

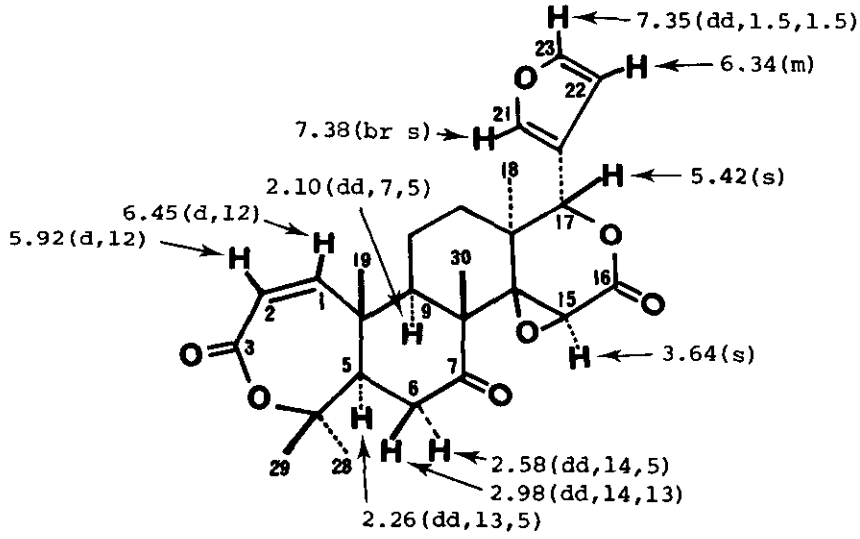
uv(CH_3OH): 288(ε 1,363)

ir($CHCl_3$): 1747(sh), 1735, 1717(sh), 1700
1620(C=C), 880(furan)



Harrisonin

1b:pmr data in $CDCl_3$
numerals in [ ] are shifts in ppm in (7:3) pyridine-d_5:$CDCl_3$; plus and minus signs denote upfield and downfield shifts,respectively.
Two sharp OH peaks at 3.67 and 5.05 ppm.



Obacunone

2b:pmr data in $CDCl_3$
CH_3 shifts: 1.14,1.26,1.46,1.52,1.52 ppm

Assignments are tabulated in Fig.4. The 6-one 321 nm band of 1 differs greatly from the typical ketone position of 280-300 nm, thus corroborating the presence of the unusual C-5/C-6/C-7 grouping. It should be noted that the signs of the cd cotton effects due to the ene lactone π,π^* transition are of opposite signs in harrisonin 1 (254 nm, positive) and obacunone 2 (250 nm, negative). This suggests that ring A has opposite chiralities namely, the twist between the double bond and lactone carbonyl is negative in 1 and positive in 2 [cf. G. Snatzke, Agnew. Chem. Intern. Ed., 7, 14 (1968); A. F. Beecham, Tetrahedron, 28, 5543 (1972)]. This conformation of ring A in harrisonin 1 is in line with the fact that both OH groups in 1 are strongly H-bonded, i.e., the 5α-OH is H-bonded to the ene carbonyl.

The 7-OH is H-bonded to the 6-one (ir, pmr), which shows that it is equitorial or 7β. This was in agreement with the observation that gradual addition of pyridine-d_5 to the $CDCl_3$ solution resulted in a conspicuous downfield shift of only the 15α-H pmr peak; in 100% pyridine-d_5 this signal had undergone a 0.66 ppm shift. Molecular models show that the 7β-OH (but not the 7α-substituent) and 15α-H are close-by in space.

The assignment of methyl resonances was attempted by extensive pmr and cmr measurements. However, combined PND, CW and PRFT cmr measurements along with pyridine-d_5 and NOE pmr measurements and comparison with obacunone failed to give unambiguous assignments.[8]

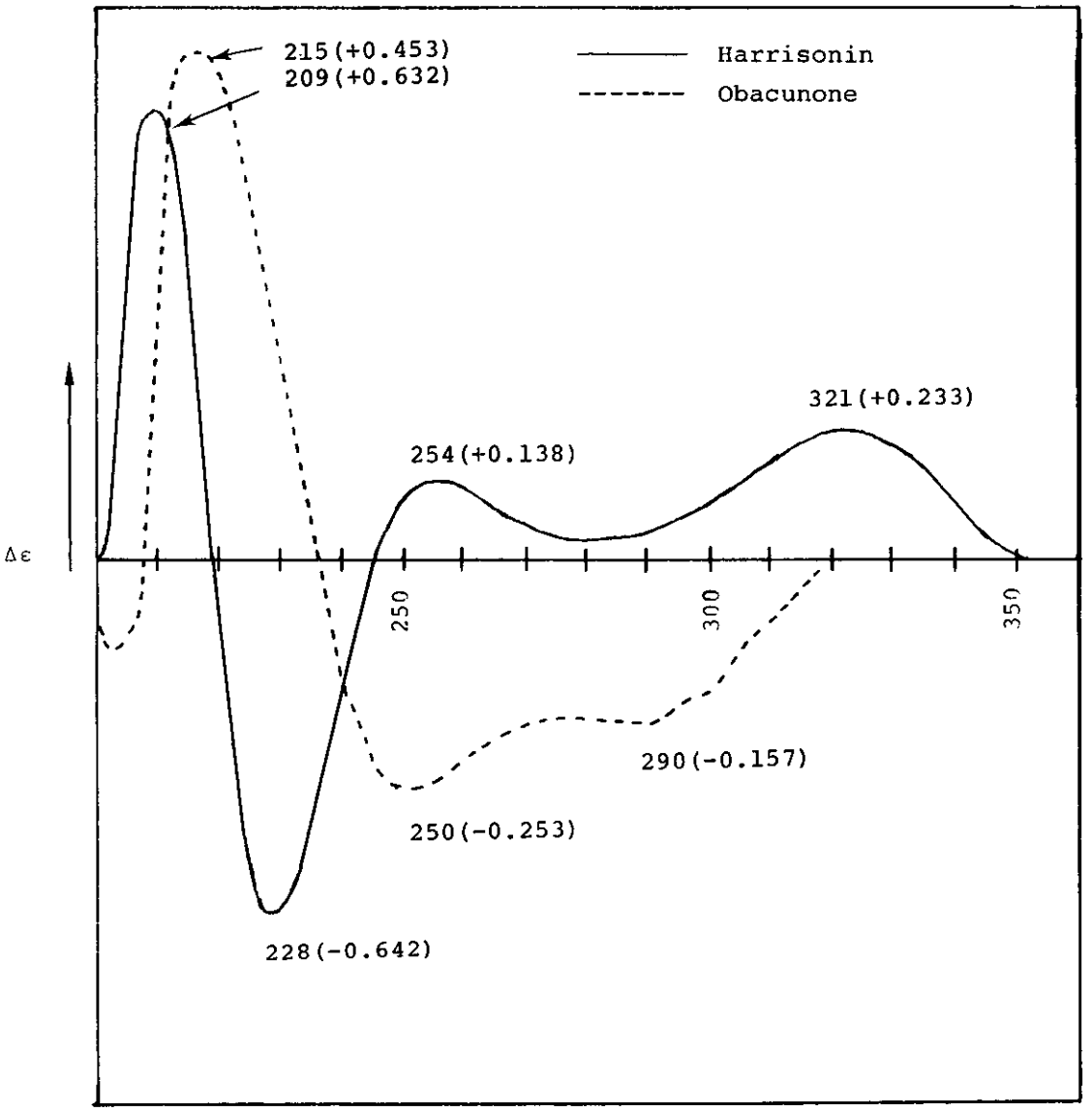


Fig.5 Cd spectra, nm(Δε), in CH_3OH

harrisonin

209(+0.632): epoxy lactone

228(-0.642): ene lactone ¶,¶*

254(+0.138): ene lactone n,¶*

321(+0.233): 6-one n,¶*

obacunone

215(+0.453): epoxy lactone
ene lactone n,¶*

250(-0.253): ene lactone ¶,¶*

290(-0.157): 7-one n,¶*

General Techniques

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Infrared spectra (ir) were determined as solutions in $CHCl_3$ on a Jasco IRA-1 grating infrared spectrophotometer. Circular dichroism spectra (cd) were recorded as solutions in CH_3OH or hexane where indicated on a Jasco J-40 spectro-polarimeter. ^1H nuclear magnetic resonance spectra (pmr) were recorded on Varian HA-100 and HR-220 spectrometers in $CDCl_3$ with tetramethylsilane as internal standard. Chemical shifts are expressed in δ units (parts per million, ppm) and coupling constants (J) in hertz. ^{13}C nuclear magnetic resonance spectra were recorded on a Jasco PS-100 spectrometer using a (180°-τ-90°-T) pulse sequence (PRFT), Jasco microprobe with a 1 mm diameter; in general 6 mg of sample was dissolved in 20 µl $CDCl_3$. Chemical shifts are expressed in δ units (parts per million, ppm), with tetramethylsilane as internal standard, and coupling constants (J) in hertz. Chemical ionization mass spectra (CI/MS), using isobutane as a carrier gas, were recorded on a Finnigan Model 3300, data system 6000.

Isolation of Harrisonin and Obacunone

The chopped root bark of Harrisonia abyssinica Oliv (Simarubaceae) (650 g) collected near Mombasa, Kenya, was extracted with 60% aqueous methanol, and the extracts were concentrated under reduced pressure. The aqueous extract was continuously extracted with ether, dried over $MgSO_4$, and evaporated under reduced pressure to give a thick oil (11.5 g). The crude extract (4.52 g) was chromatographed on 500 g silica gel (Baker 60-200) and

eluted with benzene (1.5 L) and increasing percentages of ethyl acetate in benzene (2:98, 5:95, 7:93....25:75, 1 L each). An initial fraction of 1.5 L was followed by 200 ml fractions and the fractions were monitored by thin layer chromatography (Analtech silica gel GF), developed with 10% ethyl acetate in chloroform and made visible by spraying with a solution of 2% $Ce(SO_4)_3$ in 1 N H_2SO_4 and heating until a colored spot appeared.

A. Harrisonin

Fractions 14-19 were combined and solvent removed under reduced pressure to give a yellow oil, which on addition of a small amount of 2:1 n-hexane: ether yielded 70 mg of crystals (1.65%). Combined fractions 10-30 when treated in the same manner gave smaller amounts of harrisonin, mp 155-156° (recrystallized from 2:1 n-hexane: ether); R_f 0.39; ir ($CHCl_3$) 3490, 1760(sh), 1750(sh), 1741, 1709, 1627 and 875 cm^{-1}; uv (CH_3OH) end; cd (CH_3OH) $\Delta\varepsilon_{321}$ +0.233, $\Delta\varepsilon_{254}$ +0.138, $\Delta\varepsilon_{228}$ -0.642 and $\Delta\varepsilon_{209}$ +0.632; CI/MS (isobutane) 517(MH^+), $C_{27}H_{32}O_{10}$.

B. Obacunone

Fractions 31-34 were combined and solvent removed under reduced pressure to give a fluffy yellow solid which was recrystallized by slow evaporation of a $CHCl_3$ solution to give 25 mg prisms (0.66%); mp 226-228°; R_f 0.22; ir ($CHCl_3$) 1747 (sh), 1735, 1717 (sh), 1700, 1620 and 880 cm^{-1}; uv (CH_3OH) 288 nm(1363); cd (CH_3OH) $\Delta\varepsilon_{290}$ -0.15, $\Delta\varepsilon_{250}$ -0.253 and $\Delta\varepsilon_{215}$ +0.453; CI/MS (isobutane) 455(MH^+), $C_{26}H_{30}O_7$.

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3) Antifeedants are distinguished from repellants in the sense that the former impede insects from feeding after they have licked it whereas the latter repel the insects from approach. If left with no choice, powerful antifeedants lead to insect starvation as in the case of warburganal, etc., against S. exempta (I. Kubo, et al, manuscript submitted for publication).

4) We acknowledge Dr. M. Taniguchi for carrying out these tests.

5) We acknowledge Dr. F. J. Schmitz for taking care of these tests.

6) P. Zanno, I. Miura, K. Nakanishi, D. Elder, J. Am. Chem. Soc., 97, 1975 (1975) and references cited therein.

7) K. Nakanishi, V. P. Gullo, I. Miura, T. R. Govindachari, N. Viswanathan, J. Am. Chem. Soc., 95, 6473 (1973).

8) These studies were supported by NIH Grant AI 10187.

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