A SOUTHERN ARMY-WORM ANTIFEEDANT, 12β-ACETOXYHARRISONIN FROM AN AFRICAN SHRUB HARRISONIA ABYSSINICA

Hung-wen Liu, Isao Kubo† and Koji Nakanishi*

Department of Chemistry, Columbia University, New York, N.Y. 10027, U.S.A.

Abstract: The ether extract of Harrisonia abyssinica has yielded the limonoid 12β-acetoxyharrisonin 3 in addition to the known obacunone 1 and harrisonin 2. The two insect antifeedants (against Spodoptera), harrisonin and its 12β-acetoxy derivative, both possess a 6-keto-7-hemiketal moiety, which is not an artefact, and adopt a boat conformation for ring A; this aspect contrasts with that of obacunone which is not an army-worm antifeedant.

The shrub Harrisonia abyssinica Oliv. (Simarubaceae) is widely distributed in East Africa, and the whole plant is extensively used as folk remedies for the treatment of various diseases.[1] Preliminary tests had indicated that the crude aqueous methanol extract of the root bark collected near Mombasa, Kenya, exhibited insect antifeedant (against Spodoptera exempta or African army-worm), antimicrobial,[2] cytotoxic and plant growth inhibitory activities. Monitoring the fractionation of the ether extract of the air-dried root bark by insect antifeedant bioassays[3] against the Southern army-worm Spodoptera eridania led to the isolation of a new antifeedant 12β-acetoxyharrisonin 3; in addition to the known obacunone 1 (not an antifeedant, see Figure 1 for structures) and harrisonin 2.[4] Besides azadirachtin[5] and trichilin,[6] 12β-acetoxyharrisonin which has an activity level of 500 ppm, is one of the few antifeedants active against the voracious Southern army-worm caterpillars.

12β-Acetoxyharrisonin, $C_{29}H_{34}O_{12}$, possesses the following physical constants: m.p. 253-254 °C; CI-MS (CH_4 as carrier gas), 575 (M^++1); UV (MeOH), 290 nm (ε 1,062); CD (MeOH), 321 (Δε +0.26, 6-one nπ*), 257 (Δε +0.09,ene lactone nπ*), 228 (Δε -0.78,ene lactone ππ*) and 210 nm (Δε +0.38, epoxy lactone and acetoxy); IR ($CHCl_3$), 3480 and 3400 (intramol. H-bonded OH), 1760 (sh), 1735, 1718 (sh), 1625 (C=C) and 875 cm^{-1} (furan). The tetranortriterpenoid nature of 3 which has an α,β-epoxy-δ-lactone group in ring D and an α,β-unsaturated lactone group in ring A was clarified

† Present address: College of Natural Resources, Division of Entomology and Parasitology University of California,at Berkeley, Berkeley, CA 94720

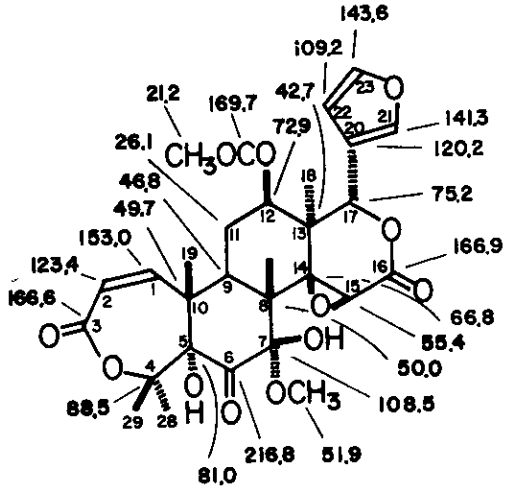
by ^{1}H-NMR and ^{13}C-NMR analyses. The ^{1}H-NMR and ^{13}C-NMR chemical shifts are shown in structures 4 and 5. Comparison of these physical data with those of harrisonin 2 indicated that the only difference between the two compounds was the presence of an additional acetoxyl group. The appearance of a CH-OAc signal at 4.75 ppm (^{1}H-NMR) and a methine peak at 72.9 ppm (^{13}C-NMR) limited the attachment of the acetoxyl group to either C-11 or C-12. The assignment of the acetoxyl group to C-12 is based on the change in chemical shifts of the adjacent carbons. Namely, the methylene (C-11) and the quaternary carbon (C-13) peaks at 15.2 and 39.6 ppm, respectively, in harrisonin were shifted downfield to 26.1 and 42.7 ppm in 3 due to the well-known acetylation or esterification effect on the β-carbon. The following ^{1}H-NMR data not only corroborates this conclusion but also leads unambiguously to a 12β-configuration: the 14 Hz and 5 Hz J values of the 9α-H 2.97 ppm signal showed that the 2.35 ppm signal ($J_{9,11}$=5 Hz) and 1.76 ppm signal ($J_{9,11}$= 14 Hz) should be assigned to the 11α(eq) and 11β(ax) protons, respectively (6, see also structure 3); since the 4.75 ppm 12-H peak is coupled to 11β-H and 11α-H by 1 Hz and 7 Hz, respectively, its configuration is α, i.e., the 12 - OAc group is β-oriented.

The two hydroxyl groups of 12β-acetoxyharrisonin are both involved in intramolecular hydrogen-bonding as evidenced from the IR bands at 3480 and 3400 cm^{-1} (in $CHCl_3$) and the two sharp ^{1}H-NMR singlets at 3.65 and 5.11 ppm (in $CDCl_3$). The CD spectrum of 12β-acetoxyharrisonin 3 is similar to that of harrisonin 2 (Figure 1), which as noted earlier,[4] has a mirror image relation to the CD of obacunone 1 in the ene-lactone ππ* (230 nm) and nπ* region (255 nm). The sign of the nπ* CD Cotton effect of ene-lactones has been correlated with the chirality between the double bond and carbonyl group, i.e., the CD and chirality have opposing signs.[7] Thus the positive nπ* bands of 2 and 3 suggest the C=C and C=O to be twisted counter-clockwise, whereas the negative band of 1 suggests the C=C/C=O chirality to be clockwise. This together with the IR evidence leads to ring A conformations as depicted in Figure 1.

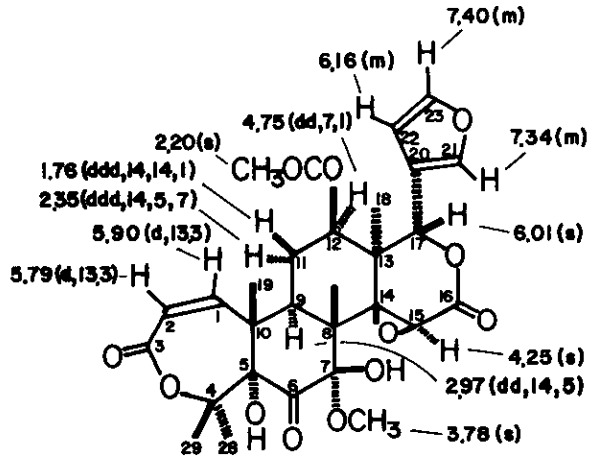
The finding that harrisonin 2 and 12β-acetoxyharrisonin 3 show similar antifeedant activities while obacunone 1 does not may be related to this difference in the conformation of ring A. That harrisonin 2 and 12β-acetoxyharrisonin 3 have been isolated from the ether extract of the root bark, and that no methanol was employed in subsequent isolation steps show that the unusual 6-keto-7-hemiketal moiety is present as such in the plant.

EXPERIMENTAL

The spectroscopic measurements were carried out with the following instruments: MS (CI and EI), Finnigan 3300, methane as carrier gas in CI-MS; UV, Cary-17; CD, JASCO J-40; IR, IBM FT-IR 85; ^{1}H-NMR, Varian HA-100; ^{13}C-NMR, JEOLCO PS-100.

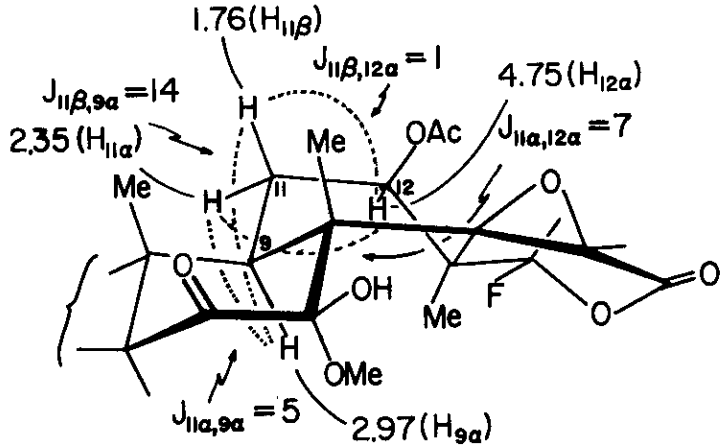


4



5

^{13}C and ^{1}H-NMR data, in $CDCl_3$, ppm from TMS.



6

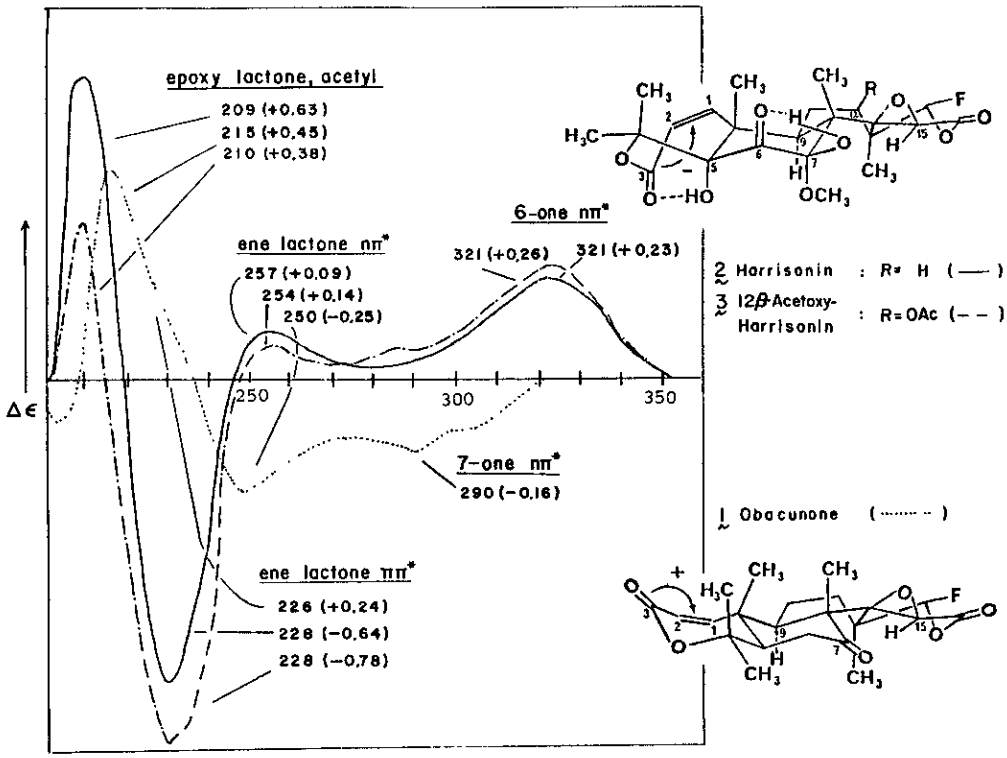


Figure 1. CD data of obacunone 1, harrisonin 2 and 12β-acetoxy-harrisonin 3 in MeOH. Intensities in parentheses are expressed in Δε.

Isolation of 12β-acetoxyharrisonin 3

The chopped root bark of Harrisonia abyssinica Oliv. (Simarubaceae) collected near Mombasa, Kenya, was extracted consecutively with solvents of increasing polarity (hexane, ether, methanol and water). Upon evaporation under reduced pressure, the ether fraction yielded a thick yellow oil. This crude ether extract (8 g) was chromatographed on 400 g of silica gel and eluted with benzene and increasing percentages of ether in benzene (2:98, 5:95, 10:90, 15:85, 20:80, 30:70 and 60:40, one liter each). Of the sixteen crude fractions collected, only fractions 4, 6, 7 and 8 showed insect antifeedant activity against the Southern army-worm Spodoptera eridania. Addition of a small amount of ether to fraction 7 (263 mg) which had been concentrated under reduced pressure led to precipitation of 12β-acetoxyharrisonin as a colorless powder. The filtrate was then separated on preparative TLC (20% ethyl acetate in benzene) to yield a second crop. The combined white solid was recrystallized from ethanol solution to give 45 mg of 12β-acetoxyharrisonin, prisms, m.p. 253-254°.

ACKNOWLEDGEMENT

The studies were supported by NIH grant AI 10187.

REFERENCES

1. J.M. Watt and M.G. Breyer-Brandwijk, 'Medicinal and Poisonous Plants of Southern and Eastern Africa,' E & S Livingston Ltd., Edinburgh and London, 1962, p. 941; J.O. Kokwaro, 'Medicinal Plants of East Africa,' East African Literature Bureau, Kampala, Nairobi, Dar es Salaam, 1976, p. 203.
2. M. Taniguchi, A. Chapya, I. Kubo and K. Nakanishi, Chem. Pharm. Bull., 1978, 26, 2910.
3. I. Kubo and K. Nakanishi, in 'Host Plant Resistance to Pests, ACS Symposium Series No. 62,' ed. by P.A. Hedin, American Chemical Society, Washington, D.C., 1977, p. 165.
4. I. Kubo, S.P. Tanis, Y.W. Lee, I. Miura, K. Nakanishi and A. Chapya, Heterocycles, 1976, 5, 485.
5. P. Zanno, I. Miura, K. Nakanishi and D. Elder, J. Am. Chem. Soc., 1975, 97, 1975.
6. M. Nakatani, J.C. James and K. Nakanishi, J. Am. Chem. Soc., 1981, 103, 1228.
7. G. Snatzke, Angew. Chem. Intern. Ed., 1968, 7, 14; A.F. Beecham, Tetrahedron, 1972, 28, 5543; G. Snatzke, 'Optical Activity and Chiral Discrimination,' ed. by S.F. Mason, D. Reidel Publishing Company, Dordrecht, Boston and London, 1978, p. 43.

Received, 16th July, 1981