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ABYSSININ, A POTENT INSECT ANTIFEEDANT FROM AN AFRICAN MEDICINAL PLANT, *BERSAMA ABYSSINICA*

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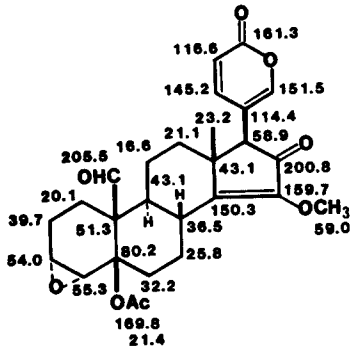
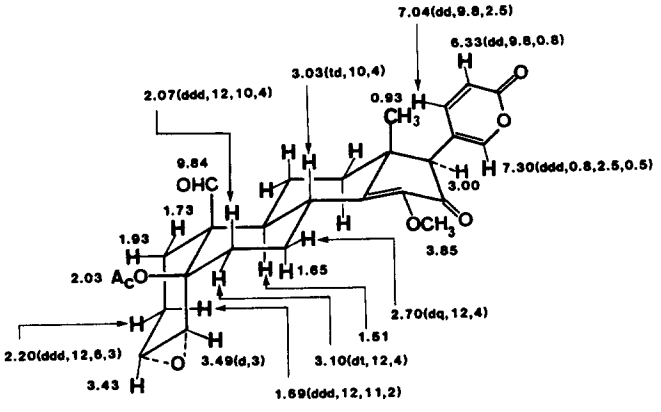
Abstract: Chemical investigation of pest insect control agents from the East African Melianthaceae plants has led to the isolation and characterization of abyssinin, a compound which exhibits very strong antifeedant activity against the cotton pest insect, Heliothis zea[1].

The root bark extract of the East African medicinal plant Bersama abyssinica (Melianthaceae)[2] has potent insect antifeedant activity against the cotton bollworm Heliothis zea, using the leaf disk bioassay with a glandless cotton cultivar[3]. Isolation of the active principle was monitored by this antifeedant bioassay. The aqueous methanol extract of 2 kg of the fresh root bark[4] was concentrated, and the residue was extracted with hexane, methylenechloride, and ethyl acetate. Silica gel chromatography of the bioactive methylenechloride extract using an ether eluent gave 200 mg of the pure active compound, abyssinin (1); mp 278°C (from ethanol). Cotton leaf disks which had been treated with 5 $\mu g/cm^2$ of abyssinin were not eaten by cotton bollworm, H. zea (PC_{95} = 5 $\mu g/cm^2$) in a 'choice' situation[5].

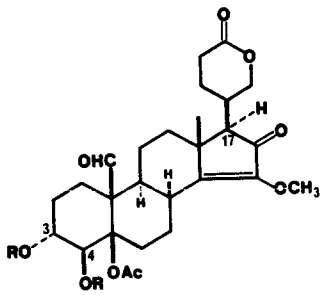
Abyssinin (1), $C_{27}H_{30}O_8$ (elemental analysis), possess the following physical constants: EI-MS, m/z 482(M^+), 454(M-CO), 439(M-$COCH_3$) and 422(M-CH_3COOH); UV (EtOH) 228(ε 7700, sh.), 256(ε 13400) and 296 nm(ε 7100, sh.); CD (EtOH) 330($\Delta\varepsilon$+3.4) and 335 nm($\Delta\varepsilon$+3.2, sh.); IR ($CHCl_3$) 2850, 1735, 1724, 1710, 1643, 1630, 1545 and 1250 cm^{-1}. The ^{13}C NMR data summarized in 1a showed the presence of three CH_3, six CH_2, five CH, three quarternary, six olefinic, and four carbonyl carbons[7]. The 400 MHz ^1H NMR data, shown in 1b, together with the other spectral data showed abyssinin to be a bufadienolide type steroid[8] containing the following groups: an acetate ester, an aldehyde, an epoxide, a quaternary methyl, a fully substituted α-methoxy enone moiety, and an α-pyrone group (λ_{max} 296 nm, ν_{max} 1710, 1630, 1545 cm^{-1}) at C-17. The three low field proton signals (7.30, 7.04[9] and 6.33 ppm) coupled to each other were assigned to the protons on α-pyrone moiety. The broad singlet proton signal at 3.00 ppm was found to be 17-H by the presence of an allylic coupling (0.5 Hz) with 21-H(7.30 ppm). A long-range coupling observed between the 0.93 ppm CH_3 and 17-H showed their zig-zag relation means this CH_3 group is located at C-13. This relationship also confirmed the configuration of the α-pyrone ring to be β, cis



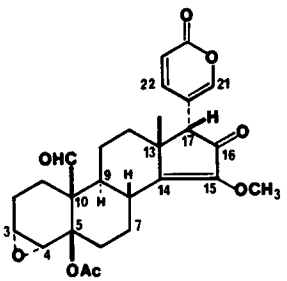
1a
[^{13}C NMR in $CDCl_3$]



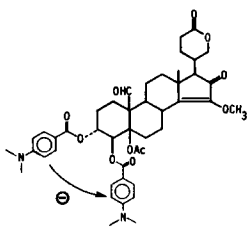
1b
[^{1}H MNR in $CDCl_3$]



2 3,4-α-epoxy
4 R=H
5 R=H, 17β-H
6 R=p-NMe_2Bz



3



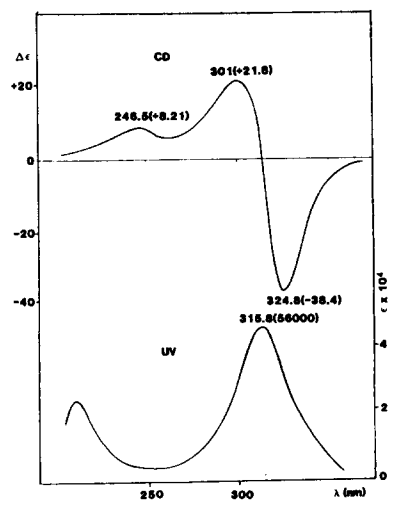


Figure 1. CD and UV spectra of abyssinin 3,4-bis(p-N,N-dimethylaminobenzoate)(6) in EtOH.

to the 13-CH_3. On the catalytic hydrogenation over 5% Pd/C in ethanol, abyssinin absorbed two moles of hydrogen to give tetrahydroabyssinin (2)[10]. The low field shift (Δ+0.22 ppm) of 13-CH_3 signal in going from 1 to 2 without any other group shifting supports the β-configuration of pyrone[11].

The α-methoxy enone moiety (λ_{max} 256 nm, ν_{max} 2850, 1710, 1643, and 1250 cm^{-1}) isolated from the continuous proton systems must be positioned at D ring on the basis of biogenetic considerations of this class of steroids. The unusual low chemical shift of the equatorial proton signal (2.70 ppm) at C-7 is due to deshielding by the through space effect of this methoxy group.

Abyssinin (1) is unstable in a protic solvent such as ethanol, and epimerizes at C-17 to give a mixture of 1 and 17-epiabyssinin (3) (3:2). A significant low field shift (Δ+0.41 ppm) of the C-13 CH_3 signal in the isolated 3 clearly showed that the configuration of pyrone ring should be α[12]. The aldehyde group and the acetate group must be located on the remained quaternary carbons, C-10(51.3 ppm) and C-5(80.2 ppm) respectively, having corresponding ^{13}C-chemical shifts. The addition of $Eu(dpm)_3$ to the $CDCl_3$ solution spread out the congested spectrum, and induced large changes of in the absorption of proton, 4-H. Since these shifts showed complexation of the lanthanide reagent with the acetate oxygen, the configuration of the epoxide was established as α.

The absolute configuration of abyssinin (1) was determined from CD studies of the tetrahydroabyssinin (2) and its 3,4-bis(p-N,N-dimethylaminobenzoate) (6). Acid catalyzed epoxy-hydrolysis of abyssinin (1) (7N H_2SO_4/THF, 50°C), followed by hydrogenation (5% Pd/C, EtOH) afforded the tetrahydroglycol (4) and its C-17 epimer (5). In general, p-N,N-dimethylaminobenzoates are prepared from by p-N,N-dimethylaminobenzoyl chloride in pyridine. But the preparation and storage of this reagent is troublesome because of its reactivity with water. Therefore we prepared a more stable reagent[13], p-N,N-dimethylaminobenzoyl nitrile, which react easily with alcohols but not with water. A solution of p-N,N-dimethylaminobenzoyl nitrile in dry CH_3CN was added to a CH_3CN solution of tetrahydroglycol (4), containing a catalytic amount of quinuclidine and this was stirred at room temperature for 17 hr. Abyssinin 3,4-bis(p-N,N-dimethylaminobenzoate) (6) was purified directly from the reaction mixture of preparative TLC. The configuration of 6 was assigned by the ^1H NMR coupling constant (J=10 Hz) of the hydrogens on carbon 3 and 4 (see Figure 1). The negative first Cotton effect (at 324.8, $\Delta\varepsilon$-38.4) in Fig. 1 showed the abolute stereochemistry of abyssinin to be that indicated in structure 1[14].

Acknowledgement. The authors are grateful to Professor A.S. Kende for 400 MHz NMR measurements, Professor N. Harada for CD measurement, and Dr. J.A. Klocke for antifeedant bioassay.

References and Notes.

1 Insects for the bioassay were kindly supplied by the agencies of the USDA in Phoenix, Az; Tifton, Ga; and Brownsville, Tx.

2 J. O. Kokwaro, "Medicinal Plants of East Africa"; East African Literature Bureau, 1976, p 159. Used as an aphrodisiac, purgative, anthelmintic and snuff for colds, and also for the treatment of dysentery, roundworm, epilepsy and haemorrhoids.

3 I. Kubo and K. Nakanishi, "Host Plant Resistance to Pests"; ed. by P. A. Hedin, ACS Symposium Series 62, American Chemical Society: Washington, D. C., 1977, pp. 165-178.

4 Collected near Kakamega, Kenya, in 1981 by one of the authors (I. Kubo).

5 I. Kubo and J. A. Klocke, l'Colloques de l'I.N.R.A., 7, 117 (1981). PC_{95}-values are concentration of abyssinin resulting in 95% protection of treated leaf disks when compared to untreated leaf disks. The artificial diet feeding assay[6] was also employed to study the effects of ingested abyssinin in a 'no choice' situation. ED_{50}-value for growth inhibition was 5 ppm against Heliothis zea. At least a part of this growth inhibitory activity of abyssinin can be attributed to an 'antifeedant' effect.

6 B. G. Chan, A. C. Waiss, W. L. Stanley and A. E. Goodban, J. Econ. Ent., 71, 366 (1978).

7 These results were based on a combination of proton-noise decoupling (PND), continuous wave decoupling (CWD), and partially relaxed Fourier transform (PRFT) techniques: P. Zanno, I. Miura, K. Nakanishi, and D. Elder, J. Am. Chem. Soc., 97, 1975 (1975).

8 P. J. May, Terpenoids and Steroids, 1, 527 (1971). L. Gsell and Ch. Tamm, Helv. Chim. Acta., 52, 551 (1969). K. Nakanishi, T. Goto, S. Ito, S. Natori and S. Nozoe, "Natural Products Chemistry"; Academic Press: New York, 1974; p 469-476. J. Meinwald, D. F. Wiemer and T. Eisner, J. Am. Chem. Soc., 101, 3055 (1979).

9 The high field shift of 22-H in comparison with those of all known bufadienolides[8] is probably due to a diamagnetic anisotropic effect, caused by C-16 carbonyl group.

10 Tetrahydroabyssinin (2), $C_{27}H_{34}O_8$, possessed the following physical properties: UV(EtOH), 248 nm(ϵ 9700); CD(10% dioxane/EtOH) 353($\Delta\epsilon$+3.4), 349 nm ($\Delta\epsilon$+3.2, sh.); EI-MS m/z 486(M^+); ^1H NMR($CDCl_3$) 9.84(1H, s, 19-H), 4.62 (1H, t, J=13 Hz, 21-H_a), 4.20(1H, dd, J=13, 4.5 Hz, 21-H_e), 3.78(3H, s, OCH_3), 3.49(1H, d, J=3.5 Hz, 4-H), 3.41(1H, br.s, 3-H), 3.09(1H,dt, J=12, 4 Hz,6-H_e), 3.02(1H, td, J=10, 4 Hz, 8-H), 2.74(1H, ddd, J=15, 9, 6 Hz, 23-H_a), 2.64(1H, dq, J=12, 4 Hz, 7-H_e), 2.48(1H, dt, J=15, 6 Hz, 23-H_e), 2.32 (1H, m, 20-H), 2.17(1H, ddd, J=12, 6, 3 Hz, 2-H_e), 2.08 (1H, m, 6-H_a), 2.03(3H, s, $OCOCH_3$), 1.87(1H, d, J=3 Hz, 17-H), 1.15(3H, s, 13-CH_3).

11 The molecular model of 1 shows that the 13-CH_3 group is situated over the pyrone ring, therefore the shift is probably due to an anisotropic effect induced by a ring current of pyrone. The sterochemistry at position 17 in 1 is consistent with those of all bufadienolides known to date.

12 17-Epiabyssinin (3) was also isolated from the same plant, B. abyssinica, and it gave a equilibrium mixture of 3 and 1 (2:3) in ethanol. But in view of the biogenetic standpoint, it is presumable that 3 is an artifact.

13 J. Goto, N. Goto, F. Shamsa, M. Sato, S. Komatsu, K. Suzaki and T. Nambara, Anal. Chim. Acta., 147, 397 (1983).

14 This also established the absolute configuration of bufadienolides isolated from the same source as antitumor agents: bersaldegenin 3-acetate and 1,3,5-orthoacetate; S. M. Kupchan and I. Ognyanov, Tetrahedron Lett., 1709 (1969), hellebrigenin 3-acetate and 3,5-diacetate; S. M. Kupchan, R. J. Hemingway and J. C. Hemingway, J. Org. Chem., 34, 3894 (1969).

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