Tetrahedron Letters,Vol.28,No.5,pp 499-502,1987
Printed in Great Britain

0040-4039/87 $3.00 + .00
Pergamon Journals Ltd.

Structure of Adenanthin

Yun-long Xu, Han-dong Sun, De-zu Wang
Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica
Kunming, Yunnan, CHINA
Takashi Iwashita, Hajime Komura
Suntory Institute for Bioorganic Research(SUNBOR)
Wakayamadai, Mishima-gun, Osaka 618, JAPAN
Mutsuo Kozuka, Keizo Naya, Isao Kubo*
Division of Entomology and Parasitology,
College of Natural Resources, University of California,
Berkeley, CA 94720, USA

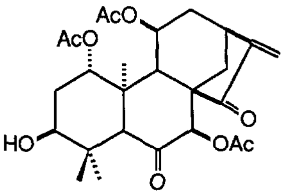
The structure of a new *ent*-kaurene diterpene, adenanthin(**1**), isolated from *Rabdosia adenantha* was established mainly by NMR experiments on the oxidized product, adenanthone, as well as on adenanthin itself. The absolute configuration of adenanthin was determined based on the cd data of di-*p*-bromobenzoyl derivatives.

A new *ent*-kaurene diterpene, adenanthin(**1**), mp. 255°(dec), $[\alpha]_D^{13}$ -76°(*c* 0.25, $CHCl_3$), was isolated in *ca.* 1% yield from the ether extract of dried aerial part of *Rabdosia adenantha* (Diels) Hara (Labiatae) collected near Yunnan, China. Purified adenanthin showed several physiological properties such as bacteriostatic, anti- inflammatory, and tumor-inhibitory activities.[1] The structure of adenanthin, including its absolute stereochemistry, was determined as **1** based on the following experimental details.

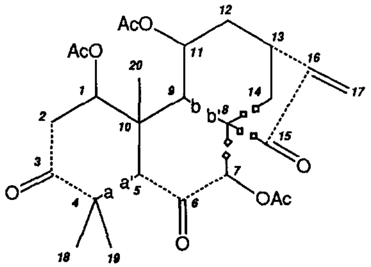
Adenanthin, $C_{26}H_{34}O_9$ (*m/z* 490, M^+; elemental analysis, Calc. C, 63.66; H, 6.99%, Obs. C, 63.73; H, 6.97%), has exhibited a typical α,β-unsaturated ketone absorption in its uv spectrum, λ_{max} 231 nm(ε 11,700, EtOH). The IR spectrum revealed that an OH(3475 cm^{-1}), an ester(1743 cm^{-1}), and a five-membered ring ketone conjugated with an exocyclic methylene(1724 and 1645 cm^{-1}) were present as functional groups.

Adenanthin possesses three acetates(δ2.27, 2.06, and 1.84 for acetyl methyls in ^{1}H-NMR, and δ170.0, 169.4, and 168.8 for carbonyls in ^{13}C-NMR, in $CDCl_3$). Examination of the connectivities of the skeletal protons using ^{1}H 2D-homonuclear shift correlation (^{1}H COSY) technique[2] and proton decoupling studies (in $CDCl_3$ and benzene-d_6) revealed several structural fragments, 1-H through 3-H, from 11-H which is vicinal to 9-H upto 14-H's, and 5-H and 20-H(10-Me) *via* C-10. It is noteworthy that the COSY spectrum suggested the presence of a long- range spin-spin coupling between geminal dimethyl protons (18- and 19-H's); irradiation of the proton at δ0.90(18-H) sharpened the δ1.31 singlet (19-H) by *ca* 0.3 Hz in its half width to prove the presence of a spin-spin coupling $^4J_{18,19}$= *ca.* 0.1 Hz.[3] Acetylation of adenanthin gave the tetraacetate **2**, $C_{28}H_{36}O_{10}$ (*m/z* 532, M^+), indicating only one OH group is present in adenanthin. A broad triplet at δ3.41($J_{2ax,3}$=2.4; $J_{2eq,3}$=3.7 Hz) in **1** was shifted downfield to δ4.66 upon acetylation, and proved that the acetylated OH group was at C-3.

In order to obtain further structural informations on a simpler spectrum, adenanthin was oxidized directly with Beckmann's mixture[4] to give a triketone, adenanthone(**3**), $C_{26}H_{32}O_9$ (*m/z* 488, M^+) quantitatively. Adenanthone is devoid of the δ3.41 signal in its ^{1}H-NMR spectrum, and the C-2 and -4 signals are shifted downfield from δ31.3 to 40.6 and from δ35.9 to 46.3, respectively, in its ^{13}C-NMR (in $CDCl_3$). ^{1}H COSY experiments of adenanthone clarified the connectivities of 1-H and 20-H(10-Me), and 20-H and 9-H presented as solid lines in Scheme 1. The long-range ^{13}C-^{1}H COSY technique[5] without broad-band decoupling in either dimension was extreamely useful in obtaining further connectivity information presented as dotted lines in Scheme 1. Much of this informaton could not be persued through ^{1}H-^{1}H COSY experiments: Quarternary sp^2 carbons were exclusively monitored using a long weak pulse.[6] The cross-sections were obtain along the proton axis at the coresponding carbons in the ^{13}C-^{1}H COSY spectrum (Fig. 1).



Adenanthin **1**



Scheme 1. Connectivities of the Structural Fragments for Adenanthone **3**.

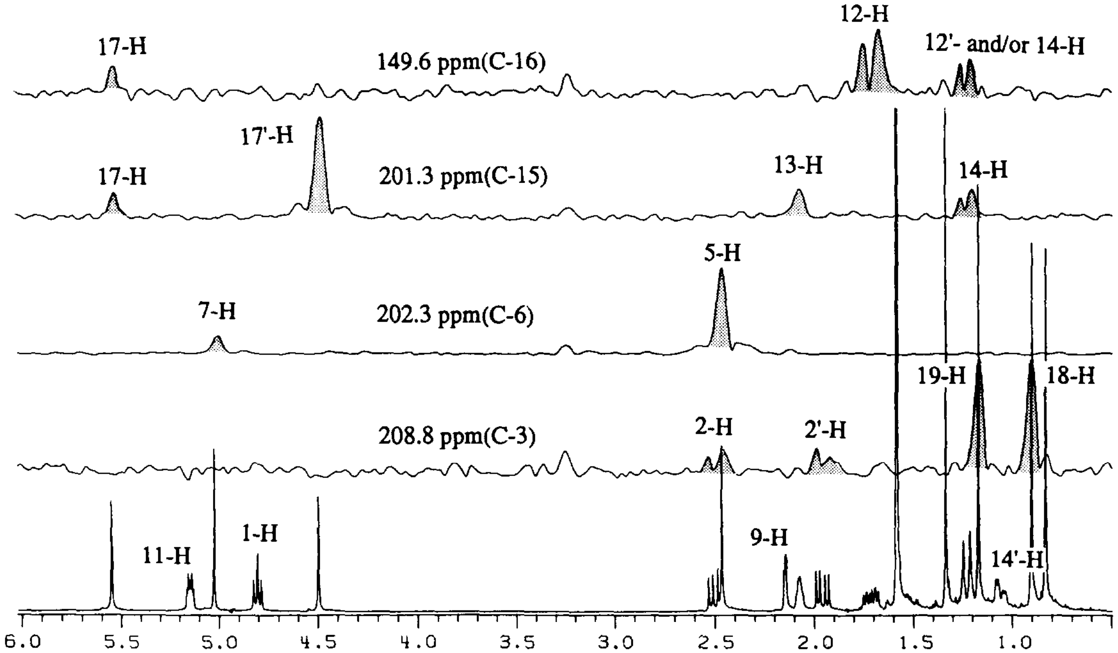


Fig. 1 Cross-sections of the Long-range ^{13}C-^{1}H COSY Spectrum of Adenanthone(**3**) at sp^2 quarternary carbons (in C_6D_6).

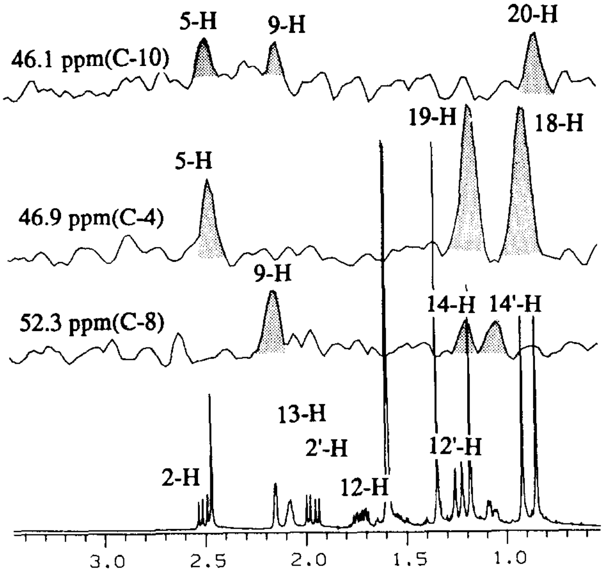


Fig. 2 Cross-sections of the Long-range ^{13}C-^{1}H COSY Spectrum of Adenanthone(3) at sp^3 quarternary carbons (in C_6D_6).

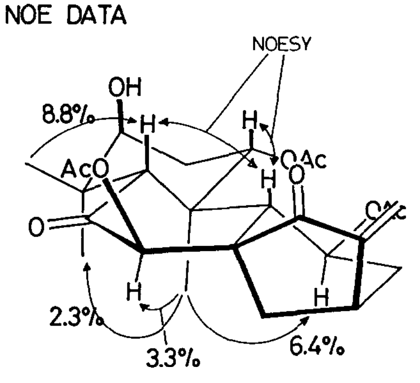
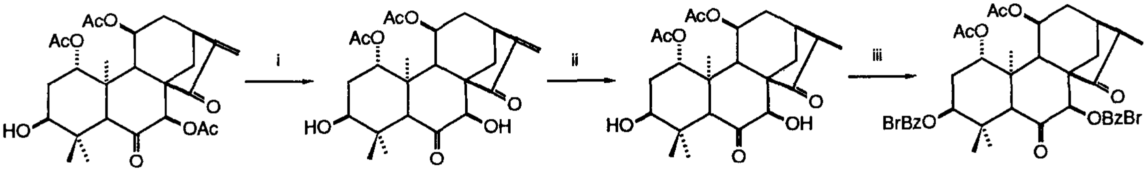


Fig. 3 NOe Studies of Adenanthin(1) (in $CDCl_3$).

The remaining bonds were connected by the same technique applied on the sp^3 quarternary carbons (Fig. 2); C-4 is coupled to 5-H (a–a') as well as to 18- and 19-H's. The last orphan carbon C-8 is coupled from 9-H (b–b'). The bonds between C-8 and C-7, -14, and -15 were obvious since only one possibility was left to connect the fragment(s) into one total structure. Thus, the planar structure of adenanthone was obtained as **3**, which could lead to the structure of adenanthin. The stereochemistry of adenanthin was determined as **1** through the analyses of the coupling constants and nOe strudies including NOESY technique. (Fig. 3)

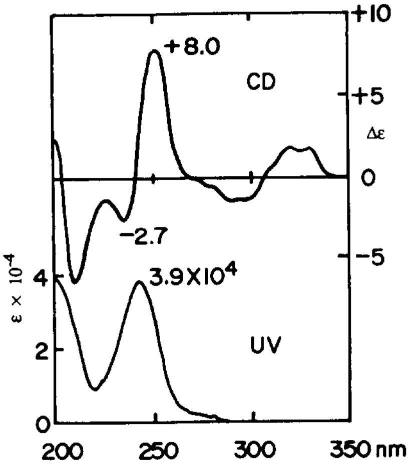
Determination of the absolute configuration of adenanthin **1** was achieved using the non-empirical cd dibenzoate chrality rule.[7] At first, 1-deacetyl-1,3-di-*p*-bromobenzoyladenanthin was prepared from 1-deacetyladenanthin obatined by a partial deacetylation reaction performed in acidic aqueous acetone. For the partial deacetylation, several reaction conditions were examined. In all cases the reaction gave a complicated mixture of compounds that had undergone a different degree of deacetylation, although this mixture seemed simpler on TLC. Finally, it was found that the reaction mixture contained adenanthin itself and 7-deacetylated adenanthin which corrapsed into single spot, and the 1-deacetylated and 1,7-di-deacetylated products which give rise to one spot even on HPTLC. Compounds with and without acetate at C-7 were not separated on an open column until these were derived into the corresponding *p*-bromobenzoates.



1

4

Scheme 2. i) H^+/Aq. Acetone; ii) H_2/Pd-C; iii) *p*-BrBzCl/Pyridine.



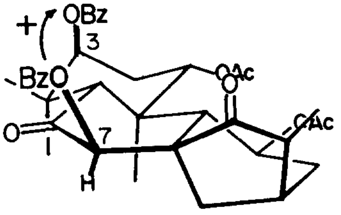


Fig. 4 UV and CD Spectra of **4** (in Hexane)(left), and the Chirality of Two Benzoates of **4**.(right).

The 1-deacetyl-1,3-dibenzoate thus obtained showed a negatively split cotton effect, $\Delta\varepsilon_{250}$ -2.1, $\Delta\varepsilon_{235}$ 0,[8] indicating the chirality of 1,3-OH's are negative, *i.e.*, an *ent*-kaurene skeleton. The interactions of the enone system in the D-ring should be considered, although it is remote from the benzoates and therefore any interaction should be small. In order to avoid any ambiguity, 16,17-dihydro-3,7-di-*p*-bromobenzoate (**4**) was prepared (Scheme 2). The coupled oscillator type positively-split cd of **4** (Fig 4)[8] proved the chirality between 3- and 7-OH groups to be positive, thus the absolute structure of adenanthin was finally determined as **1**. Another split Cotton effect due to the n-π* transition of the carbonyl groups at *ca.* 300 nm was observed. However, these transitions were not used for the interpretation of the absolute stereochemistry because other oxygen functional groups, which can also perturb the n-π* transition of the carbonyls, exist very closely in their environment.[9]

References and Notes.

1. The biological activity will be reported elsewhere.
2. Ad Bax, *"Two-dimentional Nuclear Magnetic Resonance in Liquids"*, Delft University Press, Delft, Holland, 1982.
3. Difference in the half-width of the methyl peak upon irradiation was calculated using Nicolet Line Fitting Calculation Program.
4. E. Beckmann, *Ann.* **250**, 325(1889)
5. Long-range ^{13}C-^{1}H COSY was performed using a pulse sequence of [90°(H)-t_1-90°(H)- 90°(C)-Aqc.].
6. In the usual ^{13}C-^{1}H COSY, a long delay is required for the observation of small spin-spin couplings. However, this long delay will sacrifice the *S*/*N* ratio of the obtained spectra. In order not to sacrifice the *S*/*N* ratio, the oldest version of Ernst's pulse sequence was introduced after the remodification by using a long weak pulse. Data points: 256(^{1}H) × 2K(^{13}C). For Ernst's pulse, see A.A. Maudsley, L. Müller, R.R. Ernst, *J. Magn. Reson.*, **28**, 467(1977).
7. N. Harada and K. Nakanishi, *"Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry—"*, University Science Books, Mill Valley, CA, 1983.
8. ε-Value for uv absorption at λ_{max} of the di-*p*-bromobenzoate (at 243 nm) was supposed to be 2 × 19,500, see p 34 of Ref. 7, because of the limitted amount of the sample. Δε-Values were calculated based on this ε-value.
9. Similar splitting pattern was also observable in the cd spectrum of (*16R*)-16,17-dihydro-adenanthin, which was obtained by the catalytic hydrogenation of adenanthin. Authors are grateful to Professor N. Harada, Tohoku University, for valuable discussions on this matter.

(Received in USA 11 November 1986)