Structure of Ajugarin-IV

Isao Kubo,*[a] James A. Klocke,[a] Iwao Miura,[b] and Yoshiyasu Fukuyama[b]

[a] *Division of Entomology and Parasitology, College of Natural Resources, University of California, Berkeley, California 94720, U.S.A.*

[b] *Laboratory of Natural Products Chemistry, Otsuka Pharmaceutical Co., Tokushima 771-01, Japan*

The structure of a new *neo*-clerodane diterpene, ajugarin-IV, isolated from *Ajuga remota* and possessing insecticidal activity, has been established by means of spectroscopic and chemical data.

The bitter-tasting leaves of the East African medicinal plant, *Ajuga remota* (Labiatae) are known to be naturally resistant to insect attack.[1] The diethyl ether extract of leaves of this tropical plant has recently yielded a series of new *neo*-clerodane

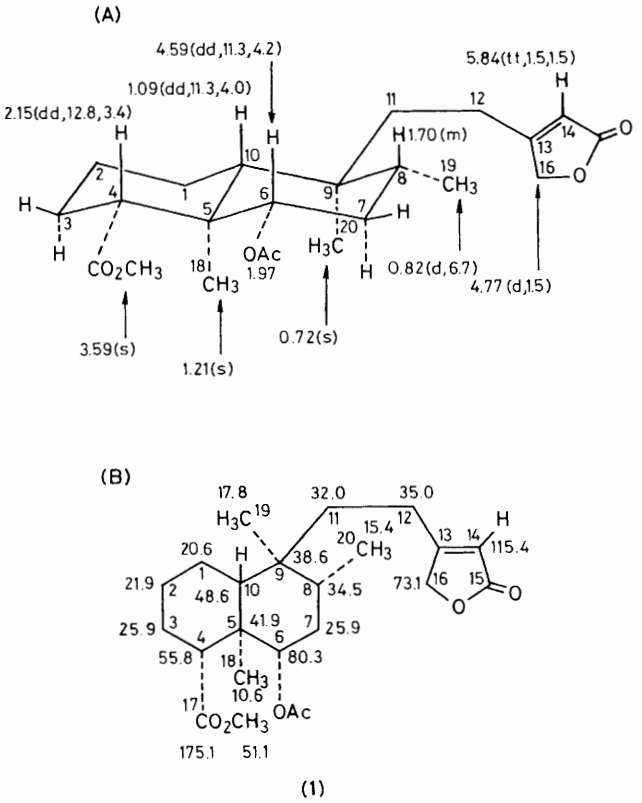
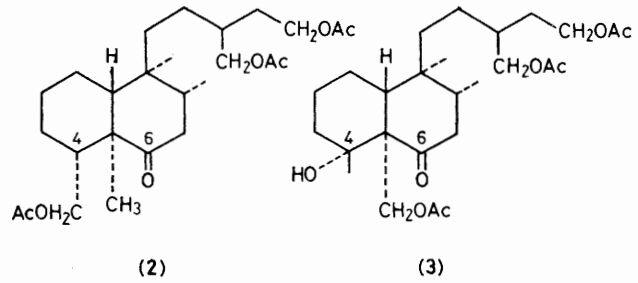


Figure 1. Ajugarin-IV. (A), ^{1}H n.m.r. data, $CDCl_3$ solution, δ values [multiplicity and J values (in Hz) in parentheses]; (B) ^{13}C n.m.r. data (δ/p.p.m.) for $CDCl_3$ solution.

diterpenoids: ajugarin-I, -II, and -III and clerodin,[2,3] having insect antifeedant activity against the African armyworm, *Spodoptera exempta* with leaf disk assay.[4] In addition to these diterpene antifeedants, two phytoecdysones, cyasterone and β-ecdysone,† have been identified in the methanol extract of the leaves and roots as possessing insect ecdysis inhibitory property against two important cotton pest insects, the fall armyworm, *Spodoptera frugiperda*, and the pink bollworm, *Pectinophora gossypiella*,[5] with an artificial diet feeding assay.[6] This feeding experiment has also led to the isolation in trace amount of another *neo*-clerodane diterpene, ajugarin-IV, as having moderate insecticidal activity.‡

Ajugarin-IV (**1**), m.p. 119—120.5 °C, $[\alpha]_D$ −57.5° (*c* 0.06, $CHCl_3$) has the following molecular formula, $C_{23}H_{34}O_6$ (M^+ 406.2356; calc. 406.2355). The presence of a conjugated γ-lactone moiety bearing an α-H as in the ajugarins is shown by the following spectral data; λ_{max}(EtOH) 215 nm (ε 17 000), ν_{max}($CHCl_3$) 1783 and 1645 cm^{-1}, ^{1}H n.m.r. δ 5.84 (1H, tt, J 1.5, 1.5 Hz, 14-H) and 4.77 (2H, d, J 1.5 Hz, 16-H), and ^{13}C n.m.r. δ 170.6 (s, C-13), 115.4 (d, C-14), 173.9 (s, C-15), and 73.1 p.p.m. (t, C-16) (Figure 1).§ The signals due to the exocyclic epoxide group found in ajugarin-I and -II were not observed in ajugarin-IV, whereas a new methoxycarbonyl group appeared at δ 3.57 (3H, s) and 175.1 (s) in the case of



ajugarin-IV. Further, the lack of a ^{13}C n.m.r. signal corresponding to C-4 in ajugarin-I and -II indicated that the epoxide ring was replaced by the methoxycarbonyl group. The stereochemistry of the methoxycarbonyl group was assigned as the α-orientation since 4-H attached to this ester group was coupled to 3α-H (J 12.8 Hz) and 3β-H (J 3.4 Hz) while showing a typical coupling pattern of axial–axial and axial–equatorial. Thus, 4-H should assume the axial β-configuration.

In addition, α-orientation of the acetoxy-group observed at ν_{max} 1730 cm^{-1} and ^{1}H n.m.r. δ 1.97 was apparent from other axial–axial and axial–equatorial coupling constants for 6-H at δ 4.95 (dd, J 11.3 and 4.2 Hz). The ^{1}H n.m.r. spectrum showed only one acetoxy-group mentioned above and did not contain the AB doublets corresponding to $-CH_2OAc$ in the ajugarins and clerodin. Instead, the presence of one extra tertiary methyl group was observed. The other remaining signals occurring in the ^{1}H and ^{13}C n.m.r. spectra were closely related to those of the ajugarins. Thus, ajugarin-IV was determined to be structure (**1**) on the basis of the aforementioned spectral evidence and by analogy with congeners isolated from *A. remota*.

The absolute configuration was determined by conversion of ajugarin-IV into the 6-oxo-derivative (**2**) by a procedure similar to that for ajugarin-I,[2] whose absolute configuration was established by *X*-ray crystallography.[3] The c.d. spectrum (MeOH) of (**2**) Δε(295 nm) −3.21 is almost identical to that of the corresponding 6-oxo-derivative of ajugarin-I (**3**).[2] Therefore, the absolute configuration of ajugarin-IV is that shown in (**1**).

Ajugarin-IV is unique in lacking both the C-4 epoxide and C-18 oxygen substituent, characteristics of the previously described antifeedant *neo*-clerodane diterpenoids. The structure–activity relationships among these *neo*-clerodane diterpenes seem to be of interest since ajugarin-IV exhibits insecticidal activity but not antifeedant property. The resistance of *A. remota* to insect attacks seems to involve an array of chemicals which have a number of adverse effects on non-adapted insect species. The chemicals include the antifeedant or insecticidal ajugarins and clerodin, and the ecdysis inhibitory phytoecdysones.

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† In addition to these two phytoecdysones, we have now isolated three more phytoecdysones by droplet counter-current chromatography. The detailed data will be published elsewhere.

‡ Ajugarin-IV exhibited insecticidal activity against silkworm, *Bombyx mori*, at 500 p.p.m. (LD_{95}), but only growth inhibitory activity against pink bollworm, *P. gossypiella* at 500 p.p.m. (ED_{50}) with artificial diet feeding experiments.

§ ^{1}H and ^{13}C N.m.r. spectra were recorded in $CDCl_3$ solution at 200 MHz and 50 MHz, respectively.