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CONFIRMATION OF THE STRUCTURE OF CALLITERPENONE, A DITERPENE FROM *CALLICARPA MACROPHYLLA**

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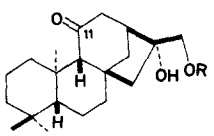
Key Word Index—*Callicarpa macrophylla*; Verbenaceae; 13β-kaurane (phyllocladane) diterpene; calliterpenone.

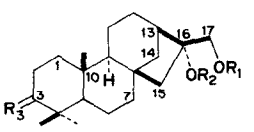
Abstract—The structure and absolute configuration of calliterpenone has been established as 3-oxo-13β-kaurane-16α,17-diol. This conclusion confirms that proposed by Ahmad and Zaman, and the formula suggested previously by Chatterjee *et al.* is revised.

INTRODUCTION

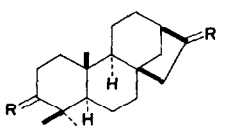
In 1972, two of the present authors [1] isolated two diterpenes, calliterpenone and its monoacetate, and assigned the structures, *ent*-11-oxokaurane-16,17-diol (**1a**) and its 17-acetate (**1b**), to them. Recently, however, Ahmad *et al.* [2] revised the structures to 3-oxo-13β-kaurane-16α, 17-diol (**2a**)



(1a) R = H
(1b) R = Ac



(2a) $R_1 = R_2 = H$, $R_3 = O$
(2b) $R_1 = Ac$, $R_2 = H$, $R_3 = O$
(5) $R_1 = R_2 = H$, $R_3 = H_2$
(6) $R_1 = Ac$, $R_2 = H$, $R_3 = H_2$
(7) $R_1 = R_2 = Ac$, $R_3 = O$
(8) $R_1 = R_2 = Ac$, $R_3 = \alpha$-H, β-OH



(3) R = O
(4) $R = H_2$

and its 17-acetate (**2b**). Our present work provides an additional evidence supporting the latter structures.

* Part 35 in the series "Terpenoids" (E. Fujita). For Part 34 see Uchida, I., Fujita, T. and Fujita, E. (1975) *Tetrahedron* **31**, 841.

RESULTS AND DISCUSSION

We confirmed the identity of our sample of calliterpenone with that of Ahmad *et al.* by direct comparison. Calliterpenone on periodate oxidation gave a diketone [1], (**3**), which on reduction [3] afforded a hydrocarbon, identical with 17-nor-13β-kaurane (**4**). Calliterpenone was reduced by Huang Minlon method [3] to yield a high yield of a diol [2], which was identical with an authentic sample of 13β-kaurane-16α,17-diol (**5**) [4,5]. Moreover, its monoacetate was proved to be identical with 13β-kaurane-16α,17-diol 17-acetate (**6**) [4].

Subsequently, the NMR of calliterpenone monoacetate in $CDCl_3$ in the presence of the shift reagent, tris-(dipivaloylmethanato)europium(III) ($Eu(DPM)_3$) was determined. Large paramagnetic shifts were found on the protons which were close to the α-hydroxy group at C-16. Thus, a multiplet at δ 6·10 (13-H), a double-doublet at δ 5·55 (*J* 5, 10 Hz, 14β-H), an AB-type signal at δ 5·29 and 4·81 (*J* 15 Hz, 15α- and 15β-H), and another AB-type signal at δ 8·66 and 8·46 (*J* 12 Hz, 17-H_2) were observed. The AB-type signals due to 15-H_2 and 17-H_2 were confirmed by the INDOR technique. The signal at δ 4·81 was assigned 15β-H by the consideration of the distance from the shift reagent (approximately from the 16-hydroxy group). Actually, 12·5% of nuclear Overhauser

effect (NOE) was observed between this proton and the methyl protons at δ 1·98 assignable to the 10-methyl group. This fact can reasonably be explained, only if calliterpenone is a 13β-kaurane-16α,17-diol derivative.

The remaining question to be determined is the location of the carbonyl function. Calliterpenone was refluxed with sodium methoxide in deuterium oxide for 1 hr to give a mixture (89% of d_2, 7% of d_1, and 4% of d_0 compounds) which was reduced with sodium borohydride in MeOH and acetylated. The NMR spectrum of the resulting diacetate in $CDCl_3$ showed a singlet signal at δ 4·48. Hence, there must be two active hydrogens adjacent to the ketone and the possible positions for the carbonyl group are limited to C-1, C-3 or C-7.

Calliterpenone diacetate, was reduced with sodium borohydride to give a mono-ol. The NMR spectrum (Table 1, A) of this alcohol showed a double-doublet signal (1H, J 7, 10 Hz) at δ 3·19 assignable to the proton on the hydroxylated carbon atom, and 13·6% of NOE was observed between this proton and a methyl protons signal at δ 0·98. Since the ring A is considered to have a chair or flattened chair conformation, the foregoing results can be rationalized by assignments of the methyl group to the 4α equatorial conformation and of the proton to the 3α axial conformation, hence of the hydroxy group to the 3β equatorial conformation. Thus, the diacetate ketone and the mono alcohol were assigned structures **7** and **8**, respectively. Accordingly, the structure of the diketone, the product from periodate oxidation of calliterpenone, was confirmed to be **3**. The NMR and mass spectra of this diketone were identical with those of McCrindle's dikteone [5].

This conclusion was supported by the following further evidence. The NMR spectrum of the alcohol (**8**) in $CDCl_3$ in the presence of the shift reagent was determined (Table 1, B). In this case, the shift reagent was added little by little, and spectra were taken after each addition. The corresponding methyl signals were thus followed by comparison with the original spectrum (A). The methyl protons signal at δ 2·35 in the final spectrum (B) and the 15β-H at δ 3·16 showed an NOE, which was observed by the INDOR technique, and hence, the former was assigned 10β-methyl group. Since an NOE was observed between the methyl protons signal at δ 4·37 and 10β-methyl protons signal, the former was assigned the 4β-methyl group. The remaining methyl signal at δ 4·24 which corresponded to the signal at δ 0·98 in the usual spectrum was reasonably assigned the 4α-methyl group.

Table 1. Protons chemical shifts* of The Alcohol **8** in 100 MHz NMR spectra

Shift reagent	Proton 3-H	4α-Me	4β-Me	10β-Me	15β-H
A Absent	3·19†	0·98	0·77	0·87	—
B Present	10·95§	4·24	4·37	2·35	3·16‡

* δ-Value from TMS as internal standard; † *dd*, J 7 and 10 Hz; § *m*; ‡ lower field *d* in AB splitting, J 15 Hz.

Table 2. Normalized shift gradients of the methyl protons influenced by the shift reagent

Compound	4β-Me	4α-Me	10β-Me
Alcohol **8***	10	9·5	4·8
Triterpene 3β-ols†	10	9·4 ~ 9·8	4·4 ~ 4·8

* 0·8 Mol equiv. of $Eu(DPM)_3$. † Ref. [6].

Buckley *et al.* [6] have used the relative induced shifts of the methyl protons as a fingerprint system for the 3-hydroxy steroids. They normalized the shift gradients ($Eu(DPM)_3$) for the fastest moving methyl to a value of 10. Application of this method gave the methyl groups of the mono alcohol **8** the values comparable to those of 3β-hydroxy steroids as shown in Table 2. These data confirmed the assignments of the methyl groups.

Consequently, calliterpenone and its monoacetate have been confirmed unambiguously to have the structure **2**a and **2**b, respectively.

The ORD curve of calliterpenone shows (+)-Cotton effect. Ourisson and his co-workers [7] described the anti-octant effect for the 8β-methyl in the 3-oxo-triterpene series, and it has been known as the 4,4,8-trimethyl effect. The observed anti-octant effect of calliterpenone in its ORD spectrum can be reasonably explained as the effect of the 15-methylene group which corresponds to 8-methyl in the 3-oxotriterpene series.

EXPERIMENTAL

Mps were taken on a micro hot-stage apparatus (Yanagimoto) and are uncorrected. IR spectra were run in KBr discs,

and NMR spectra were recorded in $CDCl_3$ with Me_4Si as internal standard, unless otherwise stated. Specific rotations were measured by an automatic polarimeter. Kieselgel (0·05–0·2 mm) and silicic acid were used for column chromatography. The TLC plates were coated with Kieselgel G.

Calliterpenone **2a**. (See Ref. [1] for detailed spectroscopic data.) Our sample was proved to be identical with that provided by Ahmad *et al.* (mmp, IR, NMR, MS, $[\alpha]_D$, and ORD) ORD $[\phi]_{589} = 163·7°$, $[\phi]_{309} = 2727·6°$ (peak), $[\phi]_{282} = 0°$, $[\phi]_{270} = -545·5°$ (trough).

Huang Minlon–Nagata reduction of diketone **3**. Calliterpenone was oxidized with periodate to give diketone **3** [1]. A mixture of **3** (10 mg), 80% N_2H_4 hydrate (212 mg), N_2H_4 dihydrochloride (50 mg) and triethylene glycol (1 g) was heated at 130° for 2·5 hr. After addition of KOH (80 mg), the temperature was raised gradually to 210° to distil the volatile substances, and kept at 210° for 2·5 hr. After cooling, H_2O was added and the mixture extracted with Et_2O. Usual work-up and column chromatography (*n*-hexane) of the crude product gave a crystalline material, whose recrystallization from MeOH afforded 17-nor-13β-kaurane (**4**) (3 mg), mp 84–85°. MS *m/e* 260·254 (M^+) (Calcd. for $C_{19}H_{32}$, 260·250). This compound was proved to be identical with the sample prepared from 13β-kaur-16-ene, by the following procedure (mp, mmp, IR, and MS).

Synthesis of 17-nor-13β-kaurane (**4**) *from 13β-kaur-16-ene* (phyllocladene). O_3 was passed through a soln of 13β-kaur-16-ene (20 mg) in MeOH (1 ml) and $CHCl_3$ (1·5 ml), at −50° for 1·5 hr. After removing excess O_3 by N_2, Me_2S (0·1 ml) was added, and the mixture was stirred at room temp overnight. Evaporation of the solvents *in vacuo* left an amorphous residue, which was chromatographed on Si gel column (CH_2Cl_2) to give a crystalline substance. Recrystallization from aq. EtOH gave 17-nor-13β-kauran-16-one [8] (18 mg), mp 97–98°. Its semicarbazone was subjected to Wolff–Kishner reduction [9] to yield 17-nor-13β-kaurane (**4**), mp 81–83°. MS *m/e* 260·253 (M^+) (Calcd. for $C_{19}H_{32}$, (260·250).

Huang Minlon–Nagata reduction of calliterpenone (**2a**). A mixture of calliterpenone (**2a**) (10 mg), was reduced in the same manner as described above giving the diol **5** (7 mg), mp 169–171°, $[\alpha]_D^{25} + 15°$ (*C* = 0·07, MeOH), IR ν_{max}: 3400 cm^{-1}; NMR δ (C_5D_5N): 0·78 (3H, *s*), 0·85 (6H, *s*), 3·99, 4·10 (each 1H, AB type, *J* 10 Hz, 17 H_2). MS *m/e* 306·256 (M^+) (Calcd. for $C_{20}H_{34}O_2$, 306·259). Identity with the authentic sample of 13β-kaurane-16α,17-diol was confirmed by mmp, IR, NMR, and $[\alpha]_D$.

13β-Kaurane-16α,17-diol 17-acetate (**6**). Diol **5** (6 mg) was acetylated (Ac_2O, C_5H_5N) and the product purified by chromatography on Si gel column and crystallized from light petrol to afford 13β-kaurane-16α,17-diol 17-acetate (**6**) (4 mg), mp 143–144°, $[\alpha]_D^{25} + 8°$ (*C* 0·06, $CHCl_3$). MS *m/e* 348·266 (M^+) (Calcd. for $C_{22}H_{36}O_3$, 348·270). It was proved to be identical with the authentic sample (mmp, IR, NMR, and TLC).

Deuterization of calliterpenone (**2a**). To a soln of Na (20 mg) in a mixture of MeOD (2 ml) and D_2O (2 ml) was added calliterpenone (**2a**) (20 mg), and the mixture was refluxed for 1 hr. After evaporation of solvents *in vacuo* and addition of D_2O (2 ml), the mixture was extracted with Et_2O. Usual work-up of the extract gave a crystalline product (13 mg) mp 154–155° (from EtOAc), IR ν_{max} cm^{-1}: 3420, 2100 (CD_2), 1703, NMR δ: 1·00 (3H, *s*), 1·03 (3H, *s*), 1·08 (3H, *s*), 3·64, and 3·75 (each 1H, AB type, *J* 11 Hz, 17 H_2). MS analysed this substance to be a mixture of d_0-(4%), d_1-(7%), and d_2-(89%) substituted derivatives. This mixture (20 mg) in MeOH (2 ml) was reduced with $NaBH_4$ (10 mg) under ice-cooling, and the mixture was stirred for 30 min. After neutralization (10% HCl) usual work-up including purification by chromatography on Si gel column and recrystallization from EtOAc yielded colorless needles (15 mg), mp 218–219°, IR ν_{max} cm^{-1}: 3410, 2190, and 2110, NMR δ (C_5D_5N): 0·93 (3H, *s*), 1·03 (3H, *s*), 1·22 (3H, *s*), 3·43 (1H, *s*, 3-H), 4·01, and 4·13 (each 1H, AB type, *J* 10 Hz, 17 H_2). This compound (7 mg) was acetylated as before and the product after purification gave colorless needles (6 mg), mp 208–210 (from EtOAc), IR ν_{max} cm^{-1}: 3400, 2200, 2120, 1720, and 1250, NMR δ: 0·90 (3H, *s*), 0·87 (6H, *s*), 2·03 (3H, *s*), 2·10 (3H, *s*), 4·18, 4·28 (each 1H, AB type, *J* 11 Hz, 17 H_2), and 4·48 (1H, *s*, 3-H).

Calliterpenone diacetate (**7**). Prepared in the usual manner (reflux), mp 151–151·5° (from MeOH), IR ν_{max} cm^{-1}: 1745, 1715, and 1255, NMR δ: 1·00 (6H, *s*), 1·07 (3H, *s*), 1·98 (3H, *s*), 2·06 (3H, *s*), 4·42, and 4·29 (each 1H, AB type, *J* 12 Hz, 17 H_2). MS *m/e* 404·254 (M^+) (calc. for $C_{24}H_{36}O_5$, 404·256). (Found: C, 70·95; H, 8·87. $C_{24}H_{36}O_5$ requires: C, 71·24; H, 8·97%).

13β-Kaurane-3β,16α,17-triol 16α,17-diacetate (**8**). A soln of **7** (35 mg) in MeOH (2 ml) was reduced with $NaBH_4$ as before giving the 3β-ol **8** (24 mg), mp 184–186° (from EtOAc), IR ν_{max} cm^{-1}: 3550, 1745, 1720, and 1265, NMR δ: 0·77 (3H, *s*), 0·87 (3H, *s*), 0·98 (3H, *s*), 1·98 (3H, *s*), 2·05 (3H, *s*), 3·19 (1H, *dd*, *J* 7 and 10 Hz, 3-H), 4·40, and 4·90 (each 1H, AB type, *J* 12 Hz, 17 H_2). MS *m/e* 346·251 (M^+-AcOH) (Calcd. for $C_{22}H_{34}O_3$, 346·249). (Found: C, 70·63; H, 9·22. $C_{24}H_{38}O_5$ requires: C, 70·88; H, 9·42%).

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