Tetrahedron. Vol. 28, pp. 4319 to 4323. Pergamon Press 1972. Printed in Great Britain

DITERPENOID CONSTITUENTS OF *CALLICARPA MACROPHYLLA* VAHL

THE STRUCTURES AND STEREOCHEMISTRY OF CALLITERPENONE AND CALLITERPENONE MONOACETATE

A. CHATTERJEE, S. K. DESMUKH and S. CHANDRASEKHARAN
Department of Chemistry, University College of Science, Calcutta-9, India

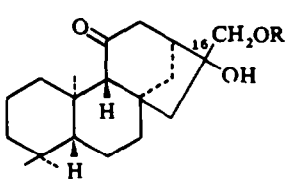
(*Received in UK* 18 *March* 1972; *Accepted for publication* 23 *March* 1972)

Abstract—From the petrol extract of the aerial parts of *Callicarpa macrophylla* Vahl (Fam: *Verbenaceae*) two new tetracyclic diterpenes *viz.* calliterpenone and calliterpenone monoacetate were isolated and their structures and stereochemistry were established as I and II respectively from a careful analysis of their spectroscopic data and chemical evidences and also by their conversions to (−)-17-norkaurane.

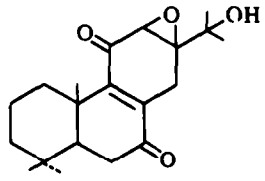
IN RECENT years tetracyclic diterpenoids in plants particularly those belonging to kaurane series have created much interest because of their biological transformation to gibberellins.[1,2] In search for new compounds of this series for biosynthetic investigations we could isolate two new tetracyclic diterpenes from *Callicarpa macrophylla* Vahl. (*Verbenaceae*), a herb growing abundantly in Bengal plain. The oil from its leaves and roots finds extensive applications in the Indian system of medicine.[3] Chromatographic resolution of the petrol extract of the aerial parts of this species has resulted in the isolation of two new kaurane derivatives which we have designated as calliterpenone (I) and calliterpenone monoacetate (II).

From chemical reactions as well as spectroscopic studies both calliterpenone and calliterpenone monoacetate were shown to be 11-oxo-kaurane derivatives and this is the first instance of 11-oxy-kauranoids known to occur in nature although callicarpone (III)[4], a Δ^8-7,11-diketone of the tricyclic diterpene series has been reported from *Callicarpa candicans*.

Calliterpenone (I), $C_{20}H_{32}O_3$ (M^+ 320), m.p. 153–5°, $[\alpha]_D^{25}$ + 36°shows in its IR spectrum a broad band in the region 3300–3400 cm^{-1} (a primary and a tertiary hydroxy functions) and a band at 1710 cm^{-1} for a cyclohexanone moiety. The NMR spectrum reveals the presence of three tertiary C–Me groups (δ 0·97, 1·01, 1·06; 3 H singlet each) while the doublets at δ 3·62 and 3·80 (J = 11 c/s) each corresponding to one proton are attributed to the methylene protons of a hydroxymethyl group (—C$\underline{H}_2$OH). A complex multiplet around δ 2·50 integrating for four protons is associated with two OH protons and two protons of a methylene group α- to the CO function (H-12). On deuteration the OH protons disappear and the spectrum shows a multiplet integrating only for two protons in this region. Calliterpenone readily forms a monoacetate (II), $C_{22}H_{34}O_4$ (M^+ 362), m.p. 124°, $[\alpha]_D^{25}$ + 23·2° in which the tertiary OH group is retained [ν_{max} 3400 (—OH), 1725, 1230 (—OCOCH$_3$), 1710 (cyclohexanone) cm^{-1}]. The NMR signal for the methylene protons does not appear as doublet as was observed in I, instead shows a sharp singlet at a lower field (δ 4·21, —C$\underline{H}_2$OCOCH$_3$). The tertiary C-methyls and acetoxy proton signals appear in the usual region (δ 0·98, 1·02, 1·07, 2·10;



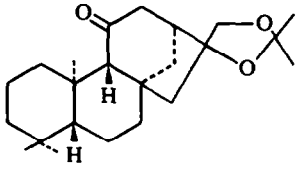
I: R = H
II: R = Ac



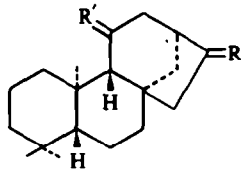
III

3 H singlet each). The OH proton (1H, s, δ 2·03) disappears on deuteration. The monoacetate (II) has been further proved to be identical (m.p., m.m.p., TLC, IR, NMR and mass spectra) with calliterpenone monoacetate isolated from the same plant source.

Compound I readily furnishes an acetonide (IV), $C_{23}H_{36}O_3$ (M^+ 360), m.p. 167° which indicates the probable presence of a 1,2 diol or a 1,3 diol system in its molecule. The ready oxidation of calliterpenone (I) to the dione (V), $C_{19}H_{28}O_2$ (M^+ 288), m.p. 178–9° subsequently confirmed the presence of the two hydroxyls in a 1,2 diol system. The dione (V) contains both a cyclopentanone and a cyclohexanone ring system as evidenced from IR spectrum (ν_{max} 1752, 1712 cm^{-1}). The same product could also be obtained by Jones' oxidation of I. The generation of a cyclopentanone moiety on oxidation leads to the inference that the grouping—C(OH)—CH_2OH must be a part of the cyclopentane ring in (I). Attempted Huang–Minlon variant of Wolff–Kishner reduction of the dione (V) failed to reduce both the CO groups. However, prolonged reduction (8 hr at 190°) yielded a monoketone (VI), $C_{19}H_{30}O$ (M^+ 274), m.p. 88–90° in which only the cyclopentanone ring is reduced (absence of band at 1750 cm^{-1}) while the



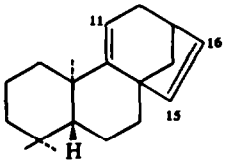
IV



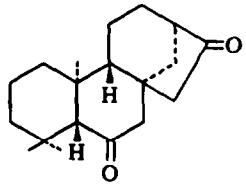
V: R = R′ = O
VI: R = H_2; R′ = O
VII: R = R′ = H, OH

6-membered ring CO is still retained (ν_{max} 1715 cm^{-1}). The failure of the 6-membered ring CO to undergo Huang–Minlon reduction even under drastic condition is most probably due to its location at C-11, a sterically hindered position. Such inert behaviour has also been observed in cases of C-11 keto steroids.[5] The site of the ketocarbonyl at C-11 could be substantiated by a series of reactions described in the sequel. The dione on reduction with $NaBH_4$ under reflux condition produces a diol (VII), $C_{19}H_{30}O_2$ (M^+ 292), m.p. 212–15°, the dehydration of which with $POCl_3$–pyridine yielded a diene (VIII). The NMR spectrum of the diene (VIII) clearly shows signals for three vinylic protons (one proton triplet at δ 5·6 and two protons in the region δ 5·8 as multiplet) two of which arising out of dehydration of C_{16}-OH. The third vinylic proton might be located at C-6 or at C-11 which observation suggests the site of the 6-membered ring CO in the dione (V) at either of these two positions. But the possibility of the occurrence of the keto function at C-6 has been excluded because the dione (IX) obtained from corymbol[6] has not been

found to be identical with the periodate oxidation product (V) of calliterpenone (I). The possibility of the occurrence of the ketocarbonyl at C-14 has also been excluded since the formation of the corresponding dehydro compound with a C-13–C-14 double bond at the bridge head would violate Bredt's rule. This could be substantiated from a Dreiding model. Obviously, the third vinylic proton in the diene (VIII) must be at C-11. This fact eventually settles the position of the keto group at C-11 in calliterpenone (I). Hydrogenation of the diene (VIII) using 5% Pd–C affords a colourless viscous oily mass which on preparative GLC yielded a pure hydrocarbon (M^+ 260). The latter was found to be identical (optical rotation, GLC and mass spectra) with (−)-17-norkaurane (X) which was prepared by Huang–Minlon reduction of an authentic sample of (−)-17-norkauran-16-one (XI)*. Since the absolute configuration of (−)-17-norkaurane is known, the absolute stereochemistry of calliterpenone and calliterpenone monoacetate are presented by XII and XIII respectively.

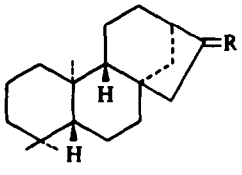


VIII

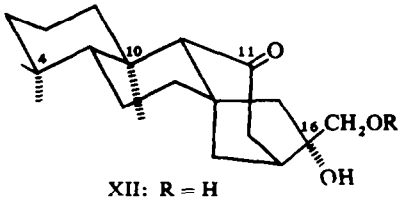


IX

The mass spectral cracking pattern of calliterpenone (I) and its derivatives are consistent with the structures assigned to these compounds. Both calliterpenone (I) and calliterpenone monoacetate (II) exhibit base peak at *m/e* 289 (M—CH_2OR; R = H, $COCH_3$ respectively). Other significant mass-peaks with their relative abundance have been reported in the Experimental.



X: R = H_2
XI: R = O



XII: R = H
XIII: R = Ac

EXPERIMENTAL

All m.ps are uncorrected. Petrol refers to light petroleum b.p. 60–80°. The IR spectra were measured with Perkin-Elmer spectrophotometer using nujol mull or chloroform as stated. NMR spectra were determined with a Varian A-60 instrument. Unless otherwise stated, the analytical samples were routinely dried at 80° over P_2O_5 for 24 hr *in vacuo*.

Isolation of calliterpenone (I) *and calliterpenone monoacetate* (II). Air dried powdered stem and leaves of *Callicarpa macrophylla* (8 kg) were exhaustively extracted with petrol for 30 hr. The petrol extract was

* Kindly provided by Professor P. R. Jefferies of the University of Western Australia, Nedlands, Australia.

concentrated (I.I.) and chromatographed over silica gel. The benzene eluates upon concentration furnished a green gummy residue (1·5 g) from which pure II (700 mg.) was obtained by crystallisation from petrol: benzene (1:1) mixture, m.p. 124°, $[\alpha]_D^{25}$ + 23·2° ($CHCl_3$), (M^+ 362), R_f 0·72 (EtOH–EtOAc = 1:3), ν_{max} (Nujol) 3400 (—OH), 1725 and 1230 (—$OCOCH_3$), 1710 (cyclohexanone) cm^{-1}; NMR signals ($CDCl_3$) at δ 4·21 (2H, s, —$C\underline{H}_2OCOCH_3$), 2·10 (3H, s, —$OCOC\underline{H}_3$), 2·03 (1H, s, —$O\underline{H}$), 0·98, 1·02 and 1·07 (3H, s, each, 3 C-methyls). In the mass spectrum, major peaks were recorded at *m/e* 362 (M^+; 9·4), 290 (21·8), 289 (100·0), 247 (7·8), 199 (9·4), 137 (6·2), 125 (7·8), 123 (6·2), 109 (9·4), 107 (7·8), 105 (6·2%) (Found: C, 71·99; H, 9·56; O, 18·25. $C_{22}H_{34}O_4$ requires: C, 72·92; H, 9·39; O, 17·68%). Upon further washing the chromatogram with benzene: chloroform (1:1) mixture, I (800 mg) migrated out and was crystallised from EtOAc, m.p. 153–5°, $[\alpha]_D^{25}$ + 36° ($CHCl_3$), (M^+ 320), R_f 0·66 (EtOH–EtOAc = 1:3), ν_{max} (KBr) 3300–3400 (broad band, OH), 1710 (cyclohexanone) cm^{-1}; NMR signals ($CDCl_3$) at δ 3·80 and 3·62 (1H, d, each, J = 11 c/s, —$C\underline{H}_2OH$), 2·50 (4H, m, two OH protons and C-12 methylene protons), 0·97, 1·01, 1·06 (3H, s, each, 3 C-methyls). Mass spectrum: peaks at *m/e* 320 (M^+; 7·4), 290 (22·2), 289 (100·0), 247 (7·4), 137 (7·4), 125 (6·4), 123 (7·4), 109 (12·9), 107 (7·4), 105 (6·4%). (Found: C, 74·56; H, 10·08; O, 15·34; $C_{20}H_{32}O_3$ requires: C, 75·0; H, 10·0; O, 15·0%).

Acetylation of calliterpenone. To calliterpenone (150 mg) in pyridine (2 ml) was added Ac_2O (5 ml) and the mixture was kept at room temp. overnight. Working up in the usual way afforded shining needles of II (120 mg), m.p. (from petrol–benzene = 1:1) 123° (M^+ 362), $[\alpha]_D^{25}$ + 23·3° ($CHCl_3$), ν_{max} (Nujol) 3400 (OH), 1728 and 1260 (OAc), 1710 (cyclohexanone) cm^{-1}. (Found: C, 71·34; H, 9·72; OAc, 16·24. $C_{22}H_{34}O_4$ requires: C, 72·92; H, 9·39; OAc, 16·3%).

Calliterpenone acetonide. To calliterpenone (100 mg) in dry acetone (20 ml) was added 2 drops of conc HCl and the mixture set aside for 24 hr. The careful evaporation of acetone furnished IV (70 mg), m.p. (from acetone) 167°; mass spectrum: peaks at *m/e* 360 (M^+; 5·3), 346 (26·3), 345 (100·0), 286 (13·2), 285 (60·6), 147 (10·5), 123 (7·9), 114 (10·5), 109 (15·8), 107 (10·5), 105 (7·9%). (Found: C, 75·86; H, 9·39. $C_{23}H_{36}O_3$ requires: C, 76·66; H, 10·00%).

Hydrolysis of II *with* 5% *alcoholic* KOH. Calliterpenone monoacetate (100 mg) was refluxed with 5% ethanolic KOH for 3 hr. Removal of solvent and extraction into $CHCl_3$ afforded fine needles of I (60 mg), m.p. (from EtOAc) 153–5°. (Found: C, 73·56; H, 9·76. $C_{20}H_{32}O_3$ requires: C, 75·0; H, 10·0%).

$NaIO_4$ *Oxidation of calliterpenone.* To I (400 mg) in MeOH (30 ml) was added $NaIO_4$ (800 mg) portionwise and the reaction mixture was kept overnight. The product was worked up by dilution and extraction into chloroform. Evaporation of solvent afforded V (250 mg), m.p. (from MeOH) 178–9°, (M^+ 288), R_f 0·78 (EtOH–EtOAc = 1:3), ν_{max} (Nujol) 1752 (cyclopentanone), 1712 (cyclohexanone) cm^{-1}. Mass spectrum: peaks at *m/e* 289 (20·5), 288 (M^+; 100·0), 245 (7·7), 233 (19·2), 231 (10·2), 203 (33·3), 202 (33·3), 190 (16·6), 189 (12·8), 177 (10·2), 163 (7·7), 125 (7·7), 119 (7·0%). (Found C, 78·87; H, 9·36. $C_{19}H_{28}O_2$ requires: C, 79·16; H, 9·72%).

Jones' oxidation of calliterpenone. To a cold soln of I (50 mg) in dry acetone (10 ml) was added Jones' reagent with continuous shaking and kept overnight at room temp. The product after usual work up yielded the dione (V) (30 mg), m.p. (from MeOH) 179–80°, (M^+ 288), R_f 0·78 (EtOH–EtOAc = 1:3), ν_{max} (Nujol) 1750 (cyclopentanone), 1710 (cyclohexanone) cm^{-1}. The compound was found to be identical with $NaIO_4$ oxidation product of calliterpenone (m.p., m.m.p., TLC, IR and Mass Spectra).

Huang–Minlon reduction of dione V. The dione V (50 mg), freshly distilled hydrazine hydrate (5 ml, 90% strength), KOH (0·70 g) and diethylene glycol (7 ml) were heated under reflux at 190° for 8 hr. The mixture was cooled, diluted with water, extracted with chloroform and dried. Removal of the solvent furnished an oil (30 mg) which was chromatographed over silica gel. The petrol-benzene (4:1) eluates yielded fine needles of VI (10 mg), m.p. (from MeOH) 88–90°, ν_{max} (Nujol) 1715 (cyclohexanone) cm^{-1}. In the mass spectrum, major peaks were recorded at *m/e* 275 (17·15), 274 (M^+; 84·2), 259 (56·1), 189 (38·6), 188 (100·0), 175 (47·4), 147 (38·6), 123 (70·1), 119 (40·3), 109 (49·1), 107 (49·1), 105 (49·1%). (Found: C, 81·99; H, 11·58. $C_{19}H_{30}O$ requires: C, 83·21; H, 10·94%).

Reduction of dione V *with* $NaBH_4$. To the dione V (150 mg) in dry MeOH (10 ml) was added $NaBH_4$ (80 mg) slowly at 0° with stirring and then refluxed for 3 hr. on steam bath. Working up in the usual way afforded the diol VII (100 mg), m.p. (from MeOH) 212–15°, ν_{max} (Nujol) 3300–3400 (OH) cm^{-1}. Mass spectrum: Major peaks at *m/e* 292 (M^+; 67·9), 274 (100·0), 259 (66·6), 147 (35·2), 137 (30·1), 136 (96·1), 135 (51·3), 123 (97·4), 122 (100·0), 121 (100·0), 120 (39·7), 119 (48·1), 109 (71·8), 107 (62·8), 105 (52·6%). (Found: C, 77·73; H, 9·98. $C_{19}H_{32}O_2$ requires: C, 78·08; H, 10·96%).

Dehydration of diol VII *to diene* VIII *with* $POCl_3$ *in Pyridine.* To VII (80 mg) dissolved in dry pyridine (5 ml) was added freshly distilled $POCl_3$ (5 ml) and the mixture refluxed at 100–110° for 10 hr. The product

was diluted, extracted with $CHCl_3$ and dried. Removal of the solvent yielded a colourless viscous oil (VIII; 40 mg); NMR signals ($CDCl_3$) at δ 5·6 (1H, t, C-11) and around δ 5·8 (2H, m, C-15, C-16).

Conversion of the diene VIII *to the hydrocarbon,* (−)-17-*norkaurane* (X). Diene VIII (40 mg) in dry MeOH (8 ml) was hydrogenated with 5% Pd–C (50 mg) for 5 hr till the absorption of H_2 was complete. The catalyst was filtered off. Evaporation of the solvent under reduced pressure yielded a syrupy liquid (20 mg). This was purified by preparative GLC using an A-350-B gas liquid chromatograph provided with a column 'P' (20% succinic acid polyester of diethylene glycol on chromosorb W/5',) maintained at 180°. The carrier gas was hydrogen. A small amount of a homogeneous mass, M^+ 260, $[\alpha]_D^{25}$ −42° ($CHCl_3$), was obtained which was characterised (optical rotation, GLC and mass-spectra) as (−)-17-norkaurane (X).

Preparation of (−)-17-*norkaurane from* (−)-17-*norkauran*-16-*one.* A mixture of XI(20 mg), freshly distilled hydrazine hydrate (1 ml, 100% strength) and KOH (0·50 g) in diethylene glycol (5 ml) was refluxed at 190–200° for 3 hr. The mixture was cooled and extracted with hexane. Evaporation of the solvent gave X (12 mg), m.p. 73°, $[\alpha]_D^{25}$ −43° ($CHCl_3$), M^+ 260 [Lit[7] m.p. 74–5°, $[\alpha]_D^{25}$ −44·1°].

Acknowledgements—Sincere thanks are accorded to Dr. B. C. Das (France), Dr. A. Hofmann (Switzerland), Dr. K. K. Chakraborty (Poona), Dr. S. C. Pakrashi (Calcutta) for spectral measurements and to Professor P. R. Jefferies (Australia) for an authentic sample of (−)-17-norkauran-16-one. Financial support from CCRIMH (India) is gratefully acknowledged.

REFERENCES

[1] J. R. Hanson and A. F. White, *J. Chem. Soc.* (C) 981 (1969)
[2] B. E. Cross, R. H. B. Galt and K. Norton, *Tetrahedron* **24**, 231 (1968)
[3] R. N. Chopra, S. L. Nayar and I. C. Chopra, *Glossary of Indian Medicinal Plants* p.45, CSIR, New Delhi (1956)
[4] K. Kawazu and T. Mitsui, *Tetrahedron Letters* No. 30, 3519 (1966)
[5] L. F. Fieser and M. Fieser, *Steroids* p. 370, Reinhold, New York (1959)
[6] M. C. Pérezamador and F. Garcia Jiménez, *Tetrahedron* **22**, 1937 (1966)
[7] M. C. Pérezamador, F. Garcia Jiménez, J. Herrán and S. E. Flores, *Ibid.* **20**, 2999 (1964)