Tetrahedron. Vol. 26, pp. 4403 to 4406. Pergamon Press 1970. Printed in Great Britain

STUDIES ON THE GENUS *PIPER*--X

STRUCTURE OF PIPOXIDE. A NEW CYCLOHEXENE EPOXIDE FROM *P. HOOKERI* LINN*

J. SINGH, K. L. DHAR and C. K. ATAL†
Regional Research Laboratory, Jammu-Tawi, India

(*Received in the UK 7 April* 1970; *Accepted for publication* 10 *June* 1970)

Abstract—Pipoxide, isolated from the leaves of *P. hookeri* L. has been shown to be 1-benzoyloxy-methyl-2-benzoyl-3-hydroxy-1,6-epoxy cyclohex-4-ene by chemical and spectroscopic evidence.

RECENTLY a new class of cyclohexene epoxide derivatives has been isolated and the identification of an antitumour compound from *P. hookeri*[1] and *P. brachystachyum*[2] reported. The isolation and structure elucidation of a new related compound pipoxide[3] from *P. hookeri* leaves, is now described.

The light petroleum extract on concentration deposited a greenish crystalline substance which on repeated purification from benzene gave a white crystalline pipoxide (I, yield 0·20%), m.p. 152–154°.

The compound $C_{21}H_{18}O_6$, M^+, 366, λ_{max}^{MeOH} 228, 274 and 280 nm has a CO group attached to a benzenoid system. The IR (KBr) indicated the presence of an OH group (3450 cm^{-1}), ester carbonyl (1730–1723 cm^{-1}), aromatic moiety (1601 cm^{-1}), benzoate (1280–1140 cm^{-1}), epoxide[4, 5] (1260, 1060 and 895 cm^{-1}) and mono-substituted benzene (710 and 682 cm^{-1}).

The NMR spectra of the compound and its derivatives are explained in Table 1.

The mass fragmentation pattern for pipoxide is explained in a manner similar to senepoxide[6] given by Hollands *et al.*

The other important peaks appeared at *m/e* 335 (M^+ —CH_2—OH), *m/e* 244 (M^+ —C_6H_5COOH), *m/e* 122 (C_6H_5—COOH), *m/e* 105 ($C_6H_5C{\equiv}O$) and *m/e* 77 (C_6H_5) which are in conformity with the proposed structure. The appearance of the peak at *m/e* 163 and *m/e* 203 suggest the epoxide linkage at C—1,6 position of the cyclohexene nucleus. Further proof of the structure was obtained by the preparation of several derivatives and their spectral data.

Hydrolysis with 10% alcoholic KOH gave benzoic acid, m.p. 122°. Treatment with 10% methanolic HCl gave a monochlorohydrin (II) $C_{21}H_{19}ClO_6$, m.p. 201–203°, M^+, 402. Its mass spectrum confirmed that the OH group is at C—1 and —Cl at C—6 position due to the presence of the peak at *m/e* 164 (2·8%) which could change to a more stable fragment *m/e* 163 (29·7%). This supplies further evidence that the epoxide is attached to C—1,6.

* For Part IX see Jagdev Singh, K. L. Dhar and C. K. Atal, *Tetrahedron Letters* 4975 (1969)

† To whom inquiries regarding this paper should be directed.

TABLE 1

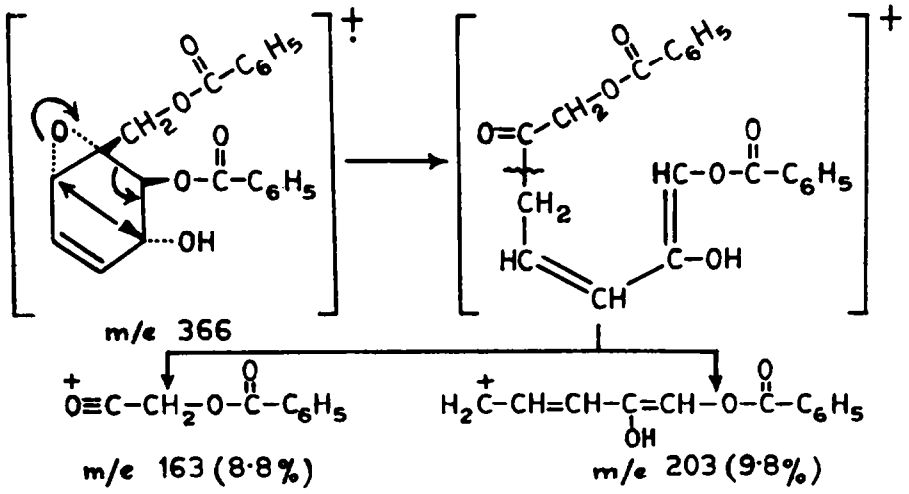
Compound	δ	No. of protons	Multiplicity	Proton assignment; see formula
1	2	3	4	5
Pipoxide (I, $CDCl_3$)	3·35	1	d, J = 6 Hz	C—3 (—OH)
	3·62	1	q, $J_{6,5}$ = 3·7 Hz, $J_{6,4}$ = 2 Hz	C—6 (—H)
	4·39[a]	1	d, $J_{3,2}$ = 8 Hz	C—3 (—H)
	4·52	1	AB d, J = 12 Hz	—O—CH_2—
	5·04	1	q, d, J = 12 Hz	
	5·75	1	d, $J_{4,5}$ = 9 Hz also allylic coupling with C—6 proton, $J_{4,6}$ = 2 Hz	C—4 (—H)
	6·06[b]	1	d, $J_{2,3}$ = 8 Hz	C—2 (—H)
	6·12	1	q, $J_{5,4}$ = 9 Hz, $J_{5,6}$ = 3·7 Hz	C—5 (—H)
	7·22–8·18	10	m	2xC_6H_5
Pipoxide chlorohydrin (II, DMSO)	4·16	1	t	C—3 (—OH)
	4·58	2	s	—O—CH_2—
	4·8	1	d	C—6 (—H)
	5·58–5·82	5	m	C—2, 3, 4, 5, (—H) and C—1 (—OH)
	7·32–8·17	10	m	2xC_6H_5
Pipoxide dihydro derivative (III, $CDCl_3$)	1·28–2·12	5	m	C—4, 5 and 6 (—H)
	3·0–3·48	1	m	C—3 (—OH)
	3·76	1	d	C—2 (—H)
	4·12	1	AB d	—O—CH_2—
	4·56	1	q d	
	5·12	1	m	C—3 (—H)
	7·28–8·14	10	m	2 × C_6H_5
Pipoxide diacetate (IV, $CDCl_3$)	2·00	3	s	C—3 and
	2·10	3	s	C—1 (—$OCOCH_3$)
	2·7	1	s	C—5 (—OH)
	4·3	2	s	—O—CH_2—
	5·55–6·1	5	m	C—2, 3, 4, 5 and 6 (—H)
	7·2–8·1	10	m	2 × C_6H_5

[a] It is a multiplet which shrinks to a doublet after D_2O treatment.

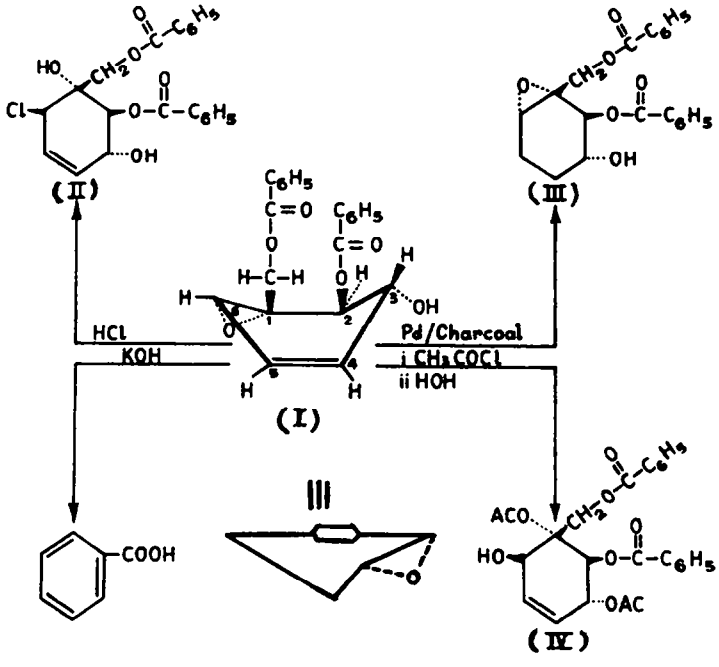
[b] The NMR ($CDCl_3$) could not give a clear coupling of C—2 and C—5 protons which was obtained as a superimposed multiplet. However, the NMR of the compound in $(CD_3)_2$ SO separated C—2 and C—5 protons.

Hydrogenation with 5% Pd—C gave a white waxy dihydro product (III), M^+, 368. The mass spectrum is in agreement with the dihydro derivative III.

Acetylation with acetyl chloride gave a white crystalline diacetate (IV), m.p. 171–172°, $C_{25}H_{24}O_{10}$, IR, 3460 cm^{-1} (—OH), 1730–1725 cm^{-1} (C=O) and 1290–1020 cm^{-1} (MeCOO— and PhCOO—). In the NMR the appearance of an OH (in



the acetate) at C—6 may be due to treatment with alkali during the process of removing excess acetyl chloride. A visible molecular ion could not be obtained but the $M^+ - 60$ peak (*m/e* 408) and other peaks at *m/e* 346 ($M^+ - 122$), *m/e* 163, *m/e* 122, *m/e* 105, *m/e* 77, *m/e* 60 and *m/e* 43 were significant.



NMR studies revealed that the proton at C—3 does not couple with the proton at C—4. From a model made for the molecule it could be seen that due to the presence of a heavy benzoate group at C—2 the preferred conformation is as shown in I with a dihedral angle between the protons of C—3 and C—4 of about 90°.

EXPERIMENTAL

M.ps are uncorrected.

Isolation of pipoxide (I). Air-dried coarsely ground leaves (1·5 kg) of *P. hookeri* were extracted in a Soxhlet with light petroleum (b.p. 60–80°) for 60 hr. The extract on concentration and cooling yielded a greenish crystalline residue (4·18 g, 0·28%) which on refluxing several times with light petroleum (b.p. 60–80°) left a light yellow crystalline material. Repeated crystallisation from benzene gave crystalline pipoxide (3·0 g, 0·2%), m.p. 152–154°, $[\alpha]_D^{20}$ +24·5 (C, 0·20, $CHCl_3$), λ_{max}^{MeOH} 228, 274 and 280 nm, M^+, 366 (mass spectrometry). (Found: C, 68·73; H, 5·31. $C_{21}H_{18}O_6$ requires: C, 68·85; H, 4·91%); IR, NMR and mass spectra described in text.

Alkaline hydrolysis of pipoxide (I). A mixture of pipoxide (250 mg) and N/2 alcoholic KOH (10 ml) was heated under reflux for 3 hr, then diluted with water (20 ml), the solvent removed *in vacuo* and extracted with $CHCl_3$ (200 ml) (fraction-A). The aqueous alkaline soln was acidified with dil HCl and extracted with ether (200 ml). The ethereal extract was washed free from mineral acid and dried over Na_2SO_4. Removal of ether yielded an acid (90 mg) crystallized from water, m.p. 122°, λ_{max}^{MeOH} 228, 273 and 281 nm and identical with benzoic acid.

The $CHCl_3$ extract (fraction-A) was dried over Na_2SO_4, the solvent removed and a light brownish syrupy liquid (110 mg) obtained as a single spot on TLC, R_f 0·64 (AcOH : water : 4:1 saturated with 80% liquid paraffin) on silica gel G impregnated with 5% soln of liquid paraffin in light petroleum (b.p. 40–60°). This could not be identified by NMR and mass spectral data.

Pipoxide chlorohydrin (II). Pipoxide (250 mg) dissolved in MeOH (5 ml) was treated with 20% methanolic HCl (5 ml). After heating under reflux on a water-bath for 3 hr, a white crystalline material separated. This was crystallized from EtOAc–benzene, m.p. 201–203°, TLC single spot, R_f 0·23 on silica gel G (EtOAc: benzene: 10:90), λ_{max}^{MeOH} 230 and 270 nm, M^+, 402, 404 (mass spectrometry). NMR and mass spectra described in text.

Hydrogenation to dihydro-pipoxide (III). Pipoxide (50 mg) in MeOH (30 ml) was hydrogenated using 10% Pd/C catalyst (20 mg) at ordinary temp and press. Absorption of H was complete after 5½ hr during which one mole of H_2 was absorbed. The catalyst was removed by filtration. Evaporation of the solvent under reduced press yielded a syrupy mass (45 mg) which on TLC gave 4 spots, the major one with R_f 0·63 (EtOAc:benzene 20:80) was separated by preparative TLC on silica gel G, into a white waxy product (25 mg). M^+, 368, *m/e* 337 ($M^+ - CH_2OH$), *m/e* 246 ($M^+ - C_6H_5CO_2H$), *m/e* 233 ($M^+ - C_6H_5CO_2CH_2$), *m/e* 122 (C_6H_5--COOH), *m/e* 105 $C_6H_5C{\equiv}O$ and *m/e* 77 C_6H_5. NMR described in the text.

Acetylation of pipoxide (I) *to its diacetate* (IV). Pipoxide (50 mg) was heated under reflux with acetyl chloride (0·5 ml) for 5 hr. The unreacted acetyl chloride was removed *in vacuo* by the addition of dry benzene. The residue was triturated with 5% Na_2CO_3 aq (5 ml), filtered, washed with water and crystallized from EtOAc–light petroleum, to give a white crystalline product (20 mg), m.p. 171–172°, TLC single spot, R_f 0·47 (EtOAc:benzene: 20:80). IR, NMR and mass spectra described in text.

Acknowledgements—We are thankful to Dr. D. H. G. Crout of the University of Exeter, Department of Chemistry, England, for supplying the microanalytical and spectral data on all the reported compounds and to Prof. P. V. Bole of St. Xavier's College, Bombay, for supply of botanical material.

* Acetylation with acetic anhydride and pyridine was unsuccessful.

REFERENCES

[1] J. Singh, K. L. Dhar and C. K. Atal, *Curr. Sci. India* **38**, 471 (1969)

[2] J. Singh and C. K. Atal, *Indian J. Pharm.* **31**, 129–130 (1969)

[3] Structure of pipoxide was presented by Dr. C. K. Atal at the 2nd Indo–Soviet Symposium *On the Chemistry of Natural Products including pharmacology*, Feb. National Institute of Sciences of India, New Delhi, India (1970)

[4] W. A. Patterson, *Analyt Chem.* **26**, 823 (1954)

[5] J. Bomstein, *Ibid.* **30**, 544 (1958)

[6] R. Hollands, D. Becher, A. Gaudemer and J. Polonsky, *Tetrahedron* **24**, 1633–50 (1968)