663. *Sesquiterpenoids. Part I. Evidence for a Nine-membered Ring in Caryophyllene.*

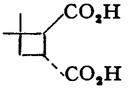
By D. H. R. Barton and A. S. Lindsey.

Evidence is presented that proves the second ring in caryophyllene to be nine-membered. Stepwise degradation limits the number of formulæ which are possible to three.

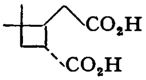
Caryophyllene, the principal sesquiterpene hydrocarbon of oil of cloves, has been the subject of numerous investigations since 1834, and a number of formulæ have been proposed (see Simonsen and Barton, " The Terpenes," Vol. III, Cambridge Univ. Press). For our knowledge of the chemistry of caryophyllene we are chiefly indebted to Simonsen and his colleagues in this country and to Ruzicka and his associates in Switzerland. It has been conclusively established that caryophyllene, $C_{15}H_{24}$, is bicyclic and contains two ethylenic linkages represented by the part expressions $\cdot CMe{:}CH\cdot$ (*a*) and ${>}C{:}CH_2$ (*b*).

A fundamental advance in our knowledge of the chemistry of caryophyllene was the recognition that vigorous oxidative degradation afforded two *cyclo*butane acids, (+)-*trans*-norcaryophyllenic acid (I) (Evans, Ramage, and Simonsen, *J.*, 1934, 1806; Ramage and Simonsen, *J.*, 1935, 532; Ruzicka and Zimmermann, *Helv. Chim. Acta*, 1935, **18**, 219), the constitution of which was confirmed by Rydon's elegant synthesis (*J.*, 1936, 593; 1937, 1340), and (+)-*trans*-caryophyllenic acid (II) (Evans, Ramage, and Simonsen, *loc. cit.*; Barton, *J. Org. Chem.*, 1950, **15**, 457), the constitution of which has also been confirmed by synthesis (Campbell and Rydon, *Chem. and Ind.*, 1951, 312; cf. Dawson and Ramage, *J.*, 1950, 3523).

The ozonolysis of caryophyllene was first studied by Semmler and Mayer (*Ber.*, 1911, **44**, 3657). The two principal products were a $C_{11}H_{18}O_3$ keto-acid and a $C_{14}H_{22}O_4$ diketo-acid.

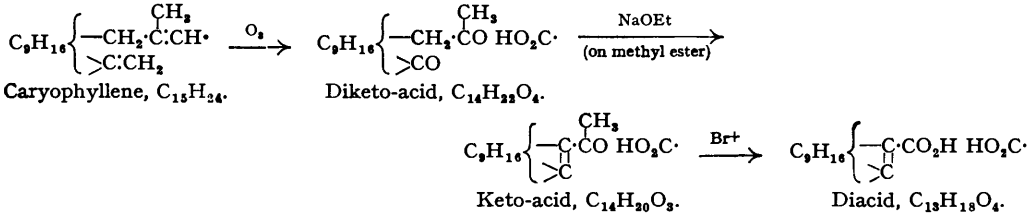


(I)



(II)

Both these acids contained one methyl ketone grouping as demonstrated by hypobromite oxidation. The reactions of the $C_{14}H_{22}O_4$ diketo-acid were investigated further by Ruzicka and Wind (*Helv. Chim. Acta*, 1931, **14**, 422) who showed that the methyl ester could be cyclised smoothly by sodium ethoxide to give an unsaturated keto-acid, $C_{14}H_{20}O_3$, which *still* contained the methyl ketone grouping, for hypobromite oxidation afforded a crystalline dicarboxylic acid, $C_{13}H_{18}O_4$, m. p. 148—149°. These changes must be interpreted in the following way :



$$C_9H_{16}\begin{cases}—CH_2\cdot\overset{CH_3}{C}{:}CH\cdot\\ {>}C{:}CH_2\end{cases}\xrightarrow{O_3}C_9H_{16}\begin{cases}—CH_2\cdot\overset{CH_3}{CO}\ HO_2C\cdot\\ {>}CO\end{cases}\xrightarrow[\text{(on methyl ester)}]{\text{NaOEt}}$$

Caryophyllene, $C_{15}H_{24}$. Diketo-acid, $C_{14}H_{22}O_4$.

$$C_9H_{16}\begin{cases}—\underset{\|}{C}\cdot\overset{CH_3}{CO}\ HO_2C\cdot\\ {>}C\end{cases}\xrightarrow{Br^+}C_9H_{16}\begin{cases}—\underset{\|}{C}\cdot CO_2H\ HO_2C\cdot\\ {>}C\end{cases}$$

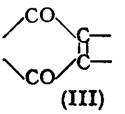
Keto-acid, $C_{14}H_{20}O_3$. Diacid, $C_{13}H_{18}O_4$.

They are of importance because they show that the double bond (*a*) (see above) must be expanded to the part expression $\cdot CH_2\cdot CMe{:}CH\cdot$.

As Ramage and Simonsen (*J.*, 1935, 1581) first emphasised, much of the early work on caryophyllene is of doubtful value because only liquid products of unauthenticated homogeneity were obtained by starting with the liquid sesquiterpene itself. They therefore employed the crystalline " β-caryophyllene nitrosite " as starting material for conversion into other crystalline substances. Another crystalline derivative, eminently suited to stepwise degradation, was reported recently by Treibs (*Chem. Ber.*, 1947, **80**, 56), who showed that autoxidation of caryophyllene furnished in good yield a crystalline caryophyllene oxide, m. p. 64°. On further oxidation by potassium permanganate this gave a crystalline oxido-ketone, $C_{14}H_{22}O_2$, m. p. 61—62°. This reaction, confirmed by transformations summarised in the sequel, demonstrates that it is double bond (*a*), not (*b*), which is involved in oxide formation.

At the outset of our researches on caryophyllene there was no experimental evidence on the number of carbon atoms separating the two ethylenic linkages. We first attempted to settle this question in the following way. Treibs's oxido-ketone, $C_{14}H_{22}O_2$, treated with hydrogen chloride in chloroform solution, gave a crystalline chlorohydrin, m. p. 145—147°, which was smoothly oxidised by chromic acid to a chloro-diketone, $C_{14}H_{21}O_2Cl$, m. p. 90°. The latter was *not* an enolisable β-diketone, as some of the formulæ previously proposed for caryophyllene would require, since it showed only low-intensity ketonic absorption at 305—309 mμ. and gave a negative ferric chloride test.

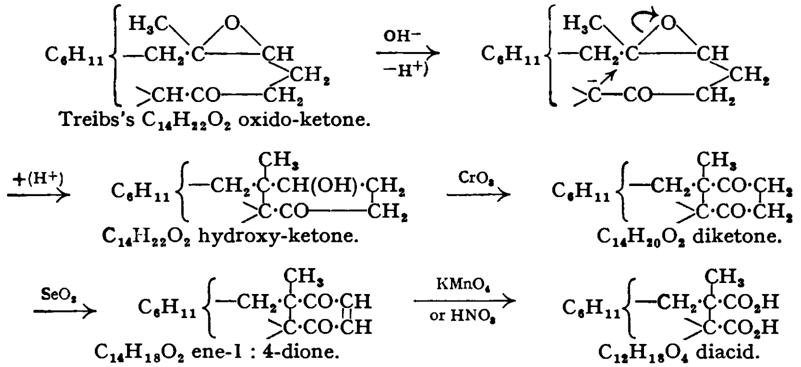
The following experiments then furnished the required information. When Treibs's $C_{14}H_{22}O_2$ oxido-ketone was refluxed with methanolic potassium hydroxide it was smoothly, and almost quantitatively, isomerised to a highly crystalline substance, m. p. 148—149°. The ultra-violet and infra-red absorption spectra showed that this compound had one carbonyl and one hydroxyl group and, further, that it was saturated and, therefore, tricyclic; moreover, it gave no colour with tetranitromethane or with the Liebermann–Burchard reagent and afforded a 2 : 4-dinitrophenylhydrazone and a 3 : 5-dinitrobenzoate. On oxidation it gave in almost quantitative yield a saturated diketone, $C_{14}H_{20}O_2$, m. p. 51—52°, characterised as the mono-2 : 4-dinitrophenylhydrazone. Oxidation of this diketone with selenium dioxide furnished a pale yellow unsaturated diketone, $C_{14}H_{18}O_2$, m. p. 47—48°, in good yield. The absorption spectrum of this compound ($\lambda\lambda_{max.}$ 221, 367, and 369 mμ., $\varepsilon = 14{,}700$, 100, and 100, respectively) showed the presence of the chromophore $-CO-\overset{|}{C}=\overset{|}{C}-CO-$ in a *cisoid*-conformation (as in III). This conclusion was confirmed by oxidation with potassium permanganate in acetone solution, or with nitric acid, which afforded in good yield a saturated bicyclic dicarboxylic acid, $C_{12}H_{18}O_4$, m. p. 168—170° (decomp.). This acid readily furnished a crystalline anhydride, m. p. 42°, merely on being melted. The formation of the C_{12} acid proves the presence of the grouping $\cdot CO\cdot CH_2\cdot CH_2\cdot CH(OH)\cdot$ in the original tricyclic hydroxy-ketone, m. p. 148—149°.



(III)

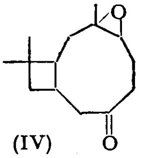
A consideration of the mechanism of genesis of this hydroxy-ketone leads to a further important conclusion. In our opinion this compound must be formed by nucleophilic attack of the ketone carbanion upon the $-C(CH_3)-$ end of the oxide ring in Treibs's $C_{14}H_{22}O_2$ oxido-ketone. Thus the C_{12} bicyclic acid must be a *succinic* acid.

These conclusions can be summarised in the following partial formulæ :



The residue, C_6H_{11}, must contain the dimethyl*cyclo*butane ring, or equivalent, and thus all the carbon atoms of caryophyllene are accounted for. It remains to discuss the fusion of the two halves of the molecule. There are three formulæ, (IV), (V), and (VI), which are formally possible as representations of Treibs's $C_{14}H_{22}O_2$ oxido-ketone. As yet, however, it is not possible to make a final decision, on the basis of our experiments, between these formulæ or between the corresponding formulæ (VII), (VIII), and (IX) for caryophyllene.

Whilst the experiments described above were in progress we received a reprint of a notable paper by Šorm, Dolejš, and Plíva (*Coll. Czech. Chem. Comm.*, 1950, **15**, 186) in which, mainly on the basis of the infra-red carbonyl frequency of Treibs's $C_{14}H_{22}O_2$ oxido-ketone, it was concluded that caryophyllene contained a nine-membered ring and was to be tentatively represented by either (VII) or (VIII). Our work is a chemical proof of the correctness of this view, and, with the reservation mentioned above, confirms the formulæ suggested for caryophyllene, which would appear to be the first natural product proved to contain a nine-membered ring. The $C_{14}H_{22}O_2$ tricyclic hydroxy-ketone, (X), (XI), or (XII) on the basis of (VII), (VIII), or (IX), respectively, would also appear to be representative of a new type of tricyclic compound.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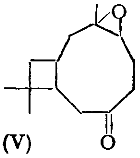
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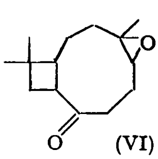
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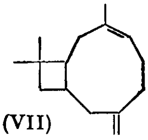
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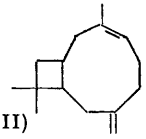
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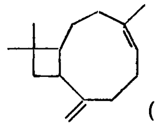
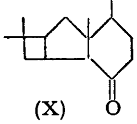

(IX)

After our preliminary report (*Chem. and Ind.*, 1951, 313) there appeared a discussion of the constitution of caryophyllene by Dawson, Ramage, and Wilson (*ibid.*, p. 464), who would appear to accept our proof of the nine-membered ring in caryophyllene, but propose for the hydrocarbon the formula (IX), which we omitted to mention in our preliminary paper, or (XIII). Of these (XIII) is, subject to the hitherto unquestioned validity of the evidence of Ruzicka and Wind (*loc. cit.*), inadmissible since it does not contain the grouping $\cdot CH_2\cdot CMe\colon CH\cdot$.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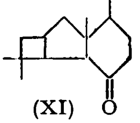
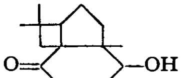

(XI)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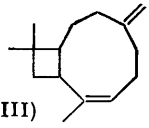
(XIII)

Experimental.

M. p.s are uncorrected. All rotations were measured in chloroform solution; the values recorded have been approximated to the nearest degree. Ultra-violet absorption spectra were, unless specified to the contrary, determined in absolute ethanol solution by use of a Unicam Spectrophotometer, Model SP 500. Infra-red measurements were kindly carried out in chloroform solution by Mr. J. L. Hales on a modified Hilger double-beam spectrometer at the D.S.I.R. Chemical Research Station, Teddington.

Light petroleum refers throughout to the fraction of b. p. 40—60°. The caryophyllene used was obtained by fractionation of clove-bud sesquiterpenes. A typical specimen had b. p. 123—125°/10 mm., n_D^{20} 1·4988, $[\alpha]_D^{20}$ −9·15° (homogeneous).

Caryophyllene Oxide.—Caryophyllene oxide was prepared without difficulty by Treibs's method (*loc. cit.*). Best purified by low-temperature crystallisation from methanol, it had m. p. 61—62°.

Oxido-ketone, $C_{14}H_{22}O_2$.—Caryophyllene oxide was oxidised by potassium permanganate according to Treibs's directions. Working up afforded the $C_{14}H_{22}O_2$ oxido-ketone, best recrystallised at low temperature from light petroleum; m. p. 62—63°, $[\alpha]_D$ −134° (*c*, 4·81), $\lambda_{max.}$ 294—297 mμ. ($\varepsilon_{max.}$ 26). The oxido-ketone was also purified by chromatography over alumina which served to separate unchanged caryophyllene oxide (more easily eluted) from the oxido-ketone (more difficultly eluted). Treatment with semicarbazide acetate in the usual way furnished the semicarbazone which, recrystallised from chloroform–ethanol, had m. p. 235—236° (Found: C, 64·3; H, 8·7. Calc. for $C_{15}H_{25}O_2N_3$: C, 64·5; H, 9·0%). For this compound Šorm, Dolejš, and Plíva (*loc. cit.*) recorded m. p. 246·5°.

Chlorohydrin from $C_{14}H_{22}O_2$ *Oxido-ketone.*—The oxido-ketone (4·1 g.) in chloroform (50 ml.) was treated with a vigorous stream of hydrogen chloride at room temperature for 15 minutes. Removal of the chloroform *in vacuo* and recrystallisation from chloroform–light petroleum furnished the highly crystalline *chlorohydrin* (2·0 g.), m. p. 145—147° (decomp.) (Found: C, 64·9; H, 9·0. $C_{14}H_{23}O_2Cl$ requires C, 64·95: H, 9·0%), which gave a strongly positive Beilstein test.

Chloro-diketone, $C_{14}H_{21}O_2Cl$.—The chlorohydrin (2·0 g.) in " AnalaR " acetic acid (25 ml.) was treated with chromium trioxide (420 mg.) dissolved in water (1 ml.) and " AnalaR " acetic acid (9 ml.), and the homogeneous solution left for 16 hours at room temperature. Working up in the usual way and filtration through alumina in benzene solution furnished the *chloro-diketone* (650 mg.) which, recrystallised from chloroform–light petroleum, had m. p. 90°, $\lambda_{max.}$ 305—309 mμ. ($\varepsilon_{max.}$ = 70) (Found: C, 65·0; H, 8·3. $C_{14}H_{21}O_2Cl$ requires C, 65·45; H, 8·25%).

Reduction of $C_{14}H_{22}O_2$ *Oxido-ketone with Lithium Aluminium Hydride.*—The oxido-ketone (5 g.) in ether (100 ml.) was refluxed with an ethereal solution of lithium aluminium hydride (0·9 g.) for 1½ hours and then left overnight. After being worked up in the usual way, the resulting *oxido-alcohol* recrystallised from chloroform–light petroleum as white needles, m. p. 140—141°, $[\alpha]_D$ −118° (*c*, 4·40)

(Found : C, 75·3; H, 11·6. $C_{14}H_{24}O_2$ requires C, 75·0; H, 10·8%). That this compound was formed by reduction of the ketone grouping and that opening of the oxide ring had not occurred was proved by chromic acid oxidation which regenerated the original oxido-ketone.

Isomerisation of the $C_{14}H_{22}O_2$ *Oxido-ketone.*—The oxido-ketone (10 g.) was refluxed with 20% (wt./vol.) methanolic potassium hydroxide (150 ml.) for 6 hours. Dilution with water, extraction with ether, etc., and recrystallisation from chloroform–light petroleum gave the highly crystalline and sparingly soluble tricyclic *hydroxy-ketone* (9·0 g.), m. p. 148—149°, $[\alpha]_D$ −32° (*c*, 4·68), $\lambda_{max.}$ 288—292 mμ. ($\varepsilon_{max.}$ = 34) (Found : C, 76·0; H, 10·0. $C_{14}H_{22}O_2$ requires C, 76·0; H, 9·85%). This compound showed a maximum absorption in the infra-red at 2·8—2·9 μ., indicative of a hydroxyl group.

Treatment of the hydroxy-ketone with 3 : 5-dinitrobenzoyl chloride in pyridine solution on the steam-bath afforded the 3 : 5-*dinitrobenzoate* which, recrystallised from benzene–light petroleum, had m. p. 154—155° (Found : C, 61·1; H, 5·8. $C_{21}H_{24}O_7N_2$ requires C, 60·6; H, 5·8%). The 2 : 4-*dinitrophenylhydrazone*, recrystallised from methanol, had m. p. 150—151° (Found : N, 14·3. $C_{20}H_{26}O_5N_4$ requires N, 13·9%).

Tricyclic Diketone, $C_{14}H_{20}O_2$.—The tricyclic hydroxy-ketone (10 g.) in "AnalaR" acetic acid (80 ml.) was treated with chromium trioxide (5·4 g.) dissolved in water (4 ml.) and "AnalaR" acetic acid (6 ml.) and set aside at room temperature for 16 hours. Working up in the usual way afforded the crystalline tricyclic *diketone* (9·5 g.), which, recrystallised from cold light petroleum, had m. p. 51—52°, $[\alpha]_D$ −173° (*c*, 1·09), $\lambda_{max.}$ 292—294 mμ. ($\varepsilon_{max.}$ 70) (Found : C, 76·3; H, 9·15. $C_{14}H_{20}O_2$ requires C, 76·2; H, 8·9%). The *mono*-2 : 4-*dinitrophenylhydrazone* was prepared in the usual way. Purified by filtration in benzene solution through alumina and recrystallisation from aqueous methanol, it had m. p. 206—207° (Found : C, 60·6; H, 6·35; N, 14·4. $C_{20}H_{24}O_5N_4$ requires C, 60·0; H, 6·05; N, 14·0%). The diketone gave no response to tests for unsaturation. The ferric chloride test was also negative.

Tricyclic Diketone, $C_{14}H_{18}O_2$.—The tricyclic diketone mentioned immediately above (20 g.) in "AnalaR" acetic acid (200 ml.) was refluxed with powdered selenium dioxide (5 g.). There was a rapid deposition of selenium, and the solution became yellow. The refluxing was continued for 1 hour. The solution was cooled, filtered, diluted with water, and extracted with ether. Washing with dilute potassium hydroxide solution caused a further precipitation of selenium. The supernatant ethereal solution was decanted off. After evaporation of the solvent *in vacuo*, a benzene solution of the residue was filtered twice through alumina. In this way a good yield of the pale yellow, crystalline, tricyclic *diketone* was obtained. Recrystallised from cold light petroleum, it had m. p. 47—48°, $[\alpha]_D$ −297° (*c*, 1·32), $\lambda_{max.}$ 221, 367, and 369 mμ. ($\varepsilon_{max.}$ = 14,700, 100, and 100, respectively) (Found : C, 76·6; H, 8·15. $C_{14}H_{18}O_2$ requires C, 77·0; H, 8·3%). A somewhat less pure specimen of the diketone had λ_{max} 221 and 348 mμ. ($\varepsilon_{max.}$ = 11,500 and 120, respectively) (Found : C, 76·45, 76·4; H, 8·6, 8·1%). For further oxidation as described below the filtration through alumina was not necessary.

Dicyclic Dicarboxylic Acid, $C_{12}H_{18}O_4$.—The crude product from the selenium dioxide oxidation of the $C_{14}H_{20}O_2$ diketone (20 g.) (see above) was dissolved in "AnalaR" acetone (400 ml.) and oxidised by addition of potassium permanganate (70 g.) dissolved in the minimum of hot water on the steam-bath. The precipitated manganese dioxide was reduced by passing in an excess of sulphur dioxide, and the oily product extracted with ether. After removal of the ether, the crude acid, which was still oily, was dissolved in "AnalaR" acetone (100 ml.) and oxidised further by addition of potassium permanganate (10 g.) in the minimum of hot water. Working up of the product as before and crystallisation from hot water to which a little methanol had been added gave crude *dicarboxylic acid* (13 g.). After further recrystallisation from the same solvent it was obtained as fine leaflets, m. p. 168—170° (decomp.), $[\alpha]_D$ −4° (*c*, 1·20) (Found : C, 63·6; H, 8·15%; equiv., 113. $C_{12}H_{18}O_4$ requires C, 63·7; H, 8·0%; equiv., 113). The m. p. depends very much on the rate of heating. This acid was saturated to tetranitromethane, to the Liebermann–Burchard reagent, and to potassium permanganate. It showed no absorption in the ultra-violet even in the range 195—215 mμ.

On melting, the $C_{12}H_{18}O_4$ dicarboxylic acid afforded, after decomposition was complete, its *anhydride*. This was purified by recrystallisation from cold light petroleum and formed beautiful needles, m. p. 42°, $[\alpha]_D$ −71° (*c*, 1·06) (Found : C, 69·0; H, 7·6%; equiv., 105, 104, 105·5. $C_{12}H_{16}O_3$ requires C, 69·2; H, 7·75%; equiv., 104). For the determination of the equivalent the anhydride was boiled with water before being titrated; in model experiments it was shown that this regenerated the dicarboxylic acid.

The $C_{12}H_{18}O_4$ dicarboxylic acid could also be prepared by boiling its precursor with concentrated nitric acid (16 ml. per g. of diketone) for 2 hours. Dilution with water, refluxing to hydrate the anhydride initially formed, and cooling gave the $C_{12}H_{18}O_4$ dicarboxylic acid (5·5 g. from 9 g. of crude diketone).

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