Constituents of Cigarette Smoke. Part X.[1] Evidence for 1,5-Hydrogen Transfer in the Formation of Non-conjugated Isoprenoid Polyolefins During Pyrolysis

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A major group of compounds present in cigarette smoke condensate are the polyolefins (I) which are not present in fresh or cured tobacco leaf. Studies on deuterium-labelled model olefins have provided evidence for specific 1,2- and 1,5-migrations of hydrogen at temperatures in the range 300—400° and suggest the role of a similar mechanism in the formation of the polyolefins (I).

AN earlier report [2] from these laboratories has described briefly the isolation of polyolefins (I; R = H, n = 1—4) from the neutral fraction of cigarette smoke condensate. The presence of these polyolefins has particular interest in view of the effects of the closely similar compound, squalene, which is reported to cause hair loss, disappearance of sebaceous glands, and the formation of tumours.[3]

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[1] Part IX. F. S. Edmunds and R. A. W. Johnstone, *J. Chem. Soc.*, 1965, 2892.

[2] D. L. Dare, I. D. Entwistle, and R. A. W. Johnstone, *Chem. and Ind.*, 1966, 629.

[3] A. I. Kosak and J. S. Swinehart, *Chem. and Ind.*, 1958, 1007.

Further, the double bonds in these olefins are readily oxidised and may lead to the formation of carcinogens such as epoxides and peroxides.[4] The formation of dipentane from solanesol (I; R = OH, $n = 7$), a major isoprenoid alcohol of tobacco leaf,[5] has been ascribed [6] to simple thermal cleavage at allylic positions in the isoprenoid chain followed by cyclisation. The same cleavage to the radical (II) could also be followed by loss of hydrogen to yield a diene, such as farnesene,[7] or by addition of hydrogen to yield the polyolefin (I). We present evidence here for the possible formation of the polyolefins by a different mechanism involving the 1,5-shift intramolecularly of hydrogen, although solanesol is still supposedly the major precursor. The isolation of the series of all-*trans*-polyolefins (I; R = H, $n = 1$—4) suggests such a common precursor in view of their absence in tobacco leaf itself. The presence of the component (I; R = H, $n = 3$), a naturally rare C_{25} polyisoprene, and the olefin (I; R = H, $n = 4$), a C_{30} polyisoprene without the central 'tail-to-tail' junction indicates a non-biogenetic origin.

After treatment of a neutral fraction [1] of cigarette smoke condensate with urea to remove long-chain paraffins, it was treated with thiourea to remove the acyclic polyisoprenoid components. The separation with thiourea was incomplete since with fewer than four isoprene units the polyolefins did not form stable inclusion complexes. The polyolefins which did not form clathrates were isolated by distillation followed by chromatography on alumina, and on argentated silica-gel. The polyolefins which had formed clathrates were isolated simply by chromatography on argentated silica-gel. Extracts of fresh and cured tobacco leaf were examined similarly but no traces of polyolefins (I; R = H, $n = 1$—4) were found.

One of the isolated olefins had a molecular weight of 274 and the mass spectrum showed the loss of C_5 units characteristic of the polyolefins (I). On hydrogenation the molecular weight increased to 282, and the resulting saturated hydrocarbon appeared to be identical in its mass spectrum and gas chromatographic retention time with phytane. The n.m.r. spectrum of the olefin showed olefinic, allylic methylene, and allylic methyl protons present in the ratios required for the polyolefin structure (I; R = H, $n = 2$). The all-*trans* nature of the molecule was shown by the position of the methyl resonances which were close to those previously reported for similar compounds.[8] The olefin was synthesised and found to be identical with the isolated material (i.r., n.m.r., mass spectral data, and gas chromatographic retention times). As a final proof of its structure, the isolated polyolefin was compared with, but found to be quite different from, the synthetic polyolefin (III) having a central 'tail-to-tail' junction, in its gas chromatographic behaviour. The isolated polyolefin yielded only laevulinic acid and no succinic acid on ozonolysis. In like manner, the other isolated polyolefins (I; R = H, $n = 1$, 3, and 4) were identified. All the polyolefins obtained from cigarette smoke were accompanied by small amounts of isomers containing terminal methylene groups, and separable by t.l.c. on argentated silica-gel. However, the amount of the isomers was too small to warrant investigation.

The polyolefin (I; R = H, $n = 4$) is isomeric with squalene, which has been reported to be present in cigarette smoke,[9] and on ozonolysis the isolated olefin did give some succinic acid. Comparison with the results of ozonolyses of squalene itself showed that the isolated polyolefin in fact must have contained 43·5% squalene. It is probable that the material termed 'iso-squalene' and previously reportedly isolated from cigarette smoke along with squalene,[9] is in fact the polyolefin (I; R = H, $n = 4$).

The olefin (I; R = H, $n = 1$) was prepared by reduction of all-*trans*-farnesyl bromide.[10] The olefin (I; R = H, $n = 2$) was obtained on treatment of all-*trans*-farnesylacetone with the phosphorylide of ethyl triphenylphosphonium bromide, and similarly the polyolefin (I; R = H, $n = 3$) was synthesised from geranylgeranylacetone.[11]

To investigate further the nature of the allylic cleavage of solanesol, we prepared a number of deuterium-labelled 5-phenylpent-2-enes (IV; R = H or Me) as model compounds in which the allylic bonds in the polyolefins were replaced by the 4,5-bond in the pentene. The model compounds were simpler to synthesise with deuterium labels than the polyolefins themselves, and one of the anticipated pyrolysis products, toluene, would be a readily isolable, stable product of pyrolysis. It was found that toluene was formed slowly from the pentenes (IV) at 300° but rapidly at 400° on heating the olefin in evacuated, sealed glass vessels. Toluene was readily identified spectroscopically in the pyrolysate and was isolated by preparative-scale gas chromatography. The deuterium content of the toluene was found by mass spectrometry, which also showed that essentially only monodeuteriotoluene was formed so that little or no exchange of the hydrogens at the 5-position of the pentenes (IV) could have occurred. The Table shows the intensities, in arbitrary units, of the ions at *m/e*

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[7] D. K. Black and F. Dickens, *Chem. and Ind.*, 1966, 197.

[8] B. C. L. Weedon, J. B. Davis, L. M. Jackman, and P. T. Siddons, *Proc. Chem. Soc.*, 1961, 261.

[9] B. L. Van Duuren and F. L. Schmitt, *Chem. and Ind.*, 1958, **32**, 1006; A. I. Kosak and J. S. Swinehart, *J. Org. Chem.*, 1960, **25**, 222; A. Rodgman, L. C. Cook, and S. S. Mims, *ibid.*, 1961, **26**, 497.

[10] O. Isler, R. Ruegg, L. Chopard-dit-Jean, H. Wagner, and K. Bernhard, *Helv. Chim. Acta*, 1956, **39**, 897.

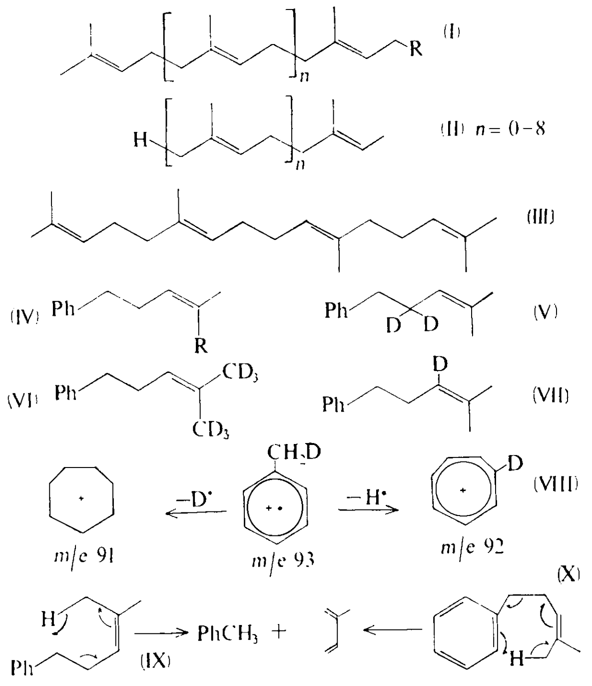
[11] O. Isler, R. Ruegg, L. H. Chopard-dit-Jean, A. Winterstein, and O. Wiss, *Helv. Chim. Acta*, 1958, **41**, 786.

[12] H. M. Grubb and S. Meyerson, 'Mass Spectrometry of Organic Ions.' ed. F. W. McLafferty, Academic Press, New York, 1963, p. 491.

93 and 92 which are, respectively, the molecular ions of the monodeuteriotoluene and toluene obtained on pyrolysis of 2-methyl-5-phenyl-4[$^2H_2$]pent-2-ene (V), 2[$^2H_3$]methyl-5-phenyl-1[$^2H_3$]pent-2-ene (VI), and 2-methyl-5-phenyl-3[^{2}H]pent-2-ene (VII). It is known[12] that in the mass spectrometer the toluene cation-radical is converted to the tropylium cation with loss of a hydrogen atom. Consequently, in the case of monodeuteriotoluene (VIII) which gives tropylium ions at *m*/*e* 92 and 91 through loss of a hydrogen or a deuterium atom, respectively, the former contribution must be subtracted from the intensity of the ion at *m*/*e* 92. The relative intensities of the ions at *m*/*e* 92 and 93 arising from monodeuteriated toluenes are in the proportion 129 : 100[12] and this factor has been used in the calculations. Thus, in the monodeuteriotoluene formed from the pentene (V), the intensity of the ion at *m*/*e* 93 is 11·0 units and the contribution this makes to the intensity of ion at *m*/*e* 92 is 11·0 × 1·29, *i.e.*, 14·2 units. Hence, the intensity of the ion at *m*/*e* 92 due to non-deuteriated toluene must be 48·0 − 14·2, *i.e.*, 33·8 units. If, the amount of 1,5-hydrogen transfer ($k_{1,5}$) is measured by the amount of non-deuteriated toluene formed, and if the amount of 1,2-hydrogen transfer ($k_{1,2}$) is measured by the amount of monodeuteriotoluene, then $k_{1,5}/k_{1,2}$ = 33·8/11 ≃ 3·1. If there had been random incorporation of deuterium into the toluene, the ratio of the ion intensities at *m*/*e* 92 and 93 would be 5·9 : 1·00 (the 4-position in the pentene was only 66% deuteriated). The incorporation of deuterium is much greater than in a random process. Similarly, from the ion intensities of the toluene isolated from the pentene (VI) we obtain $k_{1,5}/k_{1,2}$ ≃ 3·8. Random incorporation of deuterium would lead to a value of 2. The similarity of the results indicates strongly that specific hydrogen transfers occur, and in a check on the possibility of random incorporation of deuterium, the pentene (VII) was pyrolysed. In this case the incorporation of deuterium in the toluene was so low as to be within the experimental error of the method and certainly much less than random incorporation would lead to. It is concluded that at 300—400° 1,5- and 1,2-hydrogen migration (IX) can occur. It can be argued also that other shifts of hydrogen to the phenyl ring itself are involved with subsequent rearrangement of the product to toluene (X). The 1,5-transfer of hydrogen in conjugated olefins has been reported[13] but not to a saturated centre. The migrations reported here are, in fact, very like those occurring in mass spectrometry in which 1,2- and 1,5-migration of hydrogen with concomitant carbon–carbon bond cleavage are common, as in the McLafferty rearrangement.[14] During combustion in the cigarette, the all-*trans*-solanesol is subjected to temperatures varying between 100 and 900° and similar 1,2- and 1,5-hydrogen transfers could lead immediately to the polyolefins (I; R = H) found in the smoke condensate.

TABLE

Toluene from:	(V)	(VI)	(VII)
Ion intensities, *m*/*e* 93	11	33·5	1
m/*e* 92	48	52·0	82



The deuteriopentenes (V)—(VII) were synthesised by Wittig reactions with suitable component carbonyl compounds and phosphorylides. The 3-phenyl-2[$^2H_2$]-propionaldehyde used in the preparation of pentene (V) was obtained with 66% incorporation of deuterium by exchange of the α-hydrogens in 3-phenylpropionaldehyde in weakly alkaline deuterioxymethane.

EXPERIMENTAL

In all preparations involving a Wittig reaction, the phosphorylide was prepared by treating the requisite phosphonium salt (1 mol.) with n-butyl lithium (1 mol.) under nitrogen. The mixture was stirred for *ca.* 1 hr., the carbonyl compound was added, and the whole was heated under reflux for 1—3 hr. The solvent was evaporated, light petroleum (b.p. 40—60°) was added to precipitate triphenylphosphine oxide, and the resulting solution was percolated through a column of neutral alumina (Brockmann Grade I) to yield the desired olefin.

All molecular weights were determined on an A.E.I. MS 9 mass spectrometer.

5-Phenylpent-2-enes.—A Wittig reaction between acetaldehyde and 3-phenylpropyltriphenylphosphonium bromide afforded *cis*–*trans*-5-phenylpent-2-ene, $\nu_{max.}$ 970 and

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[13] W. R. Roth, *Tetrahedron Letters*, 1964, 1009; S. McLean and R. Haynes, *ibid.*, 1964, 2385; A. P. ter Boorg, H. Kloosterziel, and N. Van Meurs, *Rec. Trav. chim.*, 1963, **82**, 717, 741, 1189; E. Weth and A. S. Dreiding, *Proc. Chem. Soc.*, 1964, 59; J. Wolinski, B. Chollar, and M. D. Baird, *J. Amer. Chem. Soc.*, 1962, **84**, 2775.

[14] F. W. McLafferty, as ref. 7, p. 309.

750 cm.$^{-1}$ (Found: C, 90·5; H, 9·7%; *M*, 146. Calc. for $C_{11}H_{14}$: C, 90·4; H, 9·6%). Similarly were prepared 2[$^{2}H_3$]*methyl-5-phenyl*-1[$^{2}H_3$]*pent-2-ene* (from hexadeuterioacetone), b.p. 120°/17 mm.; $\nu_{max.}$ 850, 745, and 690 cm.$^{-1}$ (Found: C, 86·4; H + D, 13·1. $C_{12}H_{10}D_6$ requires C, 86·7; H + D, 13·3%); 2-*methyl-5-phenyl*-4[$^{2}H_2$]*pent-2-ene* (from 3-phenyl-2[$^{2}H_2$]propionaldehyde), b.p. 125°/17 mm.; $\nu_{max.}$ 745 and 695 cm.$^{-1}$ (Found: C, 88·8; H + D, 11·0. $C_{12}H_{14}D_2$ requires C, 88·8; H + D, 11·2%); 2-*methyl-5-phenyl*-3[^{2}H]*pent-2-ene* (from acetone), b.p. 98—100°/20 mm.; $\nu_{max.}$ 750 and 700 cm.$^{-1}$ (Found: C, 89·8; H + D, 10·2. $C_{12}H_{15}D$ requires C, 89·9; H + D, 10·1%).

3-*Phenyl*-1[$^{2}H_2$]*propyl bromide*. Reduction of methyl 3-phenylpropionate with lithium aluminium deuteride in ether afforded 3-phenyl-1[$^{2}H_2$]propanol which with phosphorus tribromide gave 3-phenyl-1[$^{2}H_2$]propyl bromide, b.p. 120°/20 mm.

3-*Phenyl*-2[$^{2}H_2$]*propionaldehyde*. A mixture of 3-phenylpropionaldehyde (1 g.) and anhydrous sodium [^{2}H]hydrogen carbonate (50 mg.) in deuterioxymethane (8 ml.) was shaken for 3 hr. The deuterioxymethane was removed under reduced pressure and was replaced with a fresh portion; the mixture was then shaken for a further 3 hr. After two exchanges, the aldehyde had incorporated 66% deuterium at the 2-position.

Pyrolysis of Phenylpentenes.—Samples of the olefin in glass tubes sealed *in vacuo* were heated to 300, 350, and 400° respectively for 1 hr. The contents of the tubes were examined by gas chromatography when the fraction corresponding to toluene was collected and shown to be identical with an authentic specimen. The highest yield (*ca.* 50%) of toluene was obtained at 400° and subsequent pyrolyses of the deuteriated pentenes were carried out at this temperature. The toluene obtained was examined for its deuterium content by mass spectrometry.

All-trans-3,7,11-*trimethyldodeca*-2,6,10-*triene*.—Reduction of farnesyl bromide [10] with lithium aluminium hydride gave the olefin, b.p. 80—83°/0·3 mm., $\nu_{max.}$ 822 cm.$^{-1}$; δ 1·6 (C=C–CH$_3$), 2·0 (C=C–CH$_2$), and 5·15 p.p.m. (C=C–H) (Found: C, 87·1; H, 12·9. Calc. for $C_{15}H_{26}$: C, 87·3; H, 12·7%). The all-*trans*-isomer was obtained by preparative-scale gas chromatography.

All-trans-3,7,11,15-*tetramethylhexadeca*-2,6,10,14-*tetraene*.—Farnesyl acetone [11] (5 g.) in a Wittig reaction with ethyltriphenylphosphonium bromide (7 g.) afforded a *trans*/*cis* mixture of the olefin (4 g.). A sample of the latter (0·25 g.) was added to a mixture of benzene (2·5 ml.), methanol (3·5 ml.), and recrystallised, finely ground thiourea (2 g.). The mixture was set aside at −5° for 12 hr. with frequent shaking and then filtered. The residue was washed well with light petroleum (b.p. 40—60°), dissolved in water, and extracted with light petroleum to give *all*-trans-3,7,11,15-*tetramethylhexadeca*-2,6,10,14-*tetraene* (60 mg.), b.p. 139—141°/0·35 mm., $\nu_{max.}$ 820 cm.$^{-1}$; δ 1·59, 1·88, and 5·15 p.p.m. (Found: C, 87·4; H, 12·5%; *M*, 274. $C_{20}H_{34}$ requires C, 87·5; H, 12·5%). Reduction with hydrogen over palladium–charcoal gave phytane (*M*, 282).

All-trans-3,7,11,15,19-*pentamethyleicosa*-2,6,10,14,18-*pentaene*.—A mixture of 3-hydroxy-3,7,11,15-tetramethylhexadeca-1,6,10,14-tetraene [11] (20·4 g.) in light petroleum (40 ml.), pyridine (2 ml.), and phosphorus tribromide (3 ml.) at room temperature for 3 hr. afforded 1-bromo-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene (20·0 g.). The bromo-compound was mixed with ethyl acetoacetate (12 g.), cooled to −5°, and treated dropwise during 80 min. with sodium ethoxide [sodium (1·6 g.) in ethanol (35 ml.)]. The reaction mixture was stirred at −5° for a further 2 hr. and was then set aside overnight. After addition of 10% aqueous sodium hydroxide (100 ml.) at 80° during 1 hr., the mixture was heated under reflux for 4 hr. to give 2-oxo-6,10,14,18-tetramethylnonadeca-5,9,13,17-tetraene (9 g.), b.p. 158—163°/0·05 mm. (Found: C, 83·5; H, 11·5. Calc. for $C_{23}H_{38}O$: C, 83·6; H, 11·6%). In a Wittig reaction, this ketone (7·6 g.) with ethyltriphenylphosphonium bromide (9·65 g.) yielded an olefin (6 g.) which on treatment with thiourea gave *all*-trans-3,7,11,15,19-*pentamethyleicosa*-2,6,10,14,18-*pentaene*, b.p. 140°/0·02 mm., $\nu_{max.}$ 840 cm.$^{-1}$; τ 8·32 and 8·40 (Found: C, 87·5; H, 12·3. $C_{25}H_{42}$ requires C, 87·6; H, 12·4%).

Isolation of Polyolefins from Cigarette Smoke Condensate.—The neutral fraction,[1] of the condensate from 50,000 cigarettes containing flue-cured tobacco, was chromatographed on neutral alumina (Brockmann Grade III; 2 kg.). Elution with light petroleum (40—60°) afforded a dark red oil (84 g.) which was treated twice with urea in methanol–benzene. The non-adduct forming material (72 g.) was treated twice with thiourea (400 g.) in benzene to yield an adduct (A, 9·7 g.), and a non-adducted oil (B, 60 g.). The oil (B) was distilled to give three main fractions B_1, b.p. 100—110° (8 g.), B_2, b.p. 120—160°/20 mm. (5·5 g.), and B_3, b.p. 120—160°/0·8 mm. (13·5 g.). Chromatography of fraction B_3 on alumina and argentated silica-gel gave neophytadiene [15] and an olefin (C, 119 mg.); both gave phytane on reduction. A small amount of an isomer was removed from olefin (C) by preparative t.l.c. on argentated silica-gel. Comparison with an authentic specimen showed that olefin (C) was all-*trans*-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene. Similarly, from fraction B_2 was isolated all-*trans*-3,7,11-trimethyldodeca-2,6,10-triene.

Fraction (A) was chromatographed on argentated silica-gel to yield mainly solanesene and two other major olefinic components (A_1 and A_2) of which the former (A_1) was shown to be all-*trans*-3,7,11,15,19-pentamethyleiocosa-2,6,10,14,18-pentaene. The olefin (A_2) (*M*, 410) was hydrogenated catalytically to the saturated hydrocarbon (*M*, 422). The i.r. spectrum showed only trisubstituted double bonds present ($\nu_{max.}$ 835 cm.$^{-1}$), and the n.m.r. spectrum and gas chromatographic retention times were very close to those of squalene. On ozonolysis, the olefin (A_2) gave both succinic and laevulinic acids, and from the quantity of succinic acid formed was estimated the amount of squalene in the sample.

Ozonolysis of Polyolefins.—A sample of the olefin was ozonised at −60° in methylene chloride and the ozonide was decomposed with alkaline hydrogen peroxide. The acids formed were esterified with diazomethane and separated gas chromatographically on a 120 ft. capillary column coated with polypropylene glycol.

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