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COMPONENTS OF ROYAL JELLY

10-HYDROXY-*TRANS*-DEC-2-ENOIC ACID*

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Abstract—Chemical and physical evidence is cited which proves that the olefinic linkage in 10-hydroxydec-2-enoic acid, isolated from royal jelly, has the *trans*-configuration.

ROYAL jelly[1] is believed to be the sole food administered by young workers or nurse bees of the common honey bee species *Apis mellifica* to female larvae destined to become queens. The composition of royal jelly has been intensively investigated.[1]

Townsend and Lucas[2] recognized, and Abbot and French[3] confirmed, that the major portion of the ether-soluble fraction of royal jelly comprised an optically inactive acid $C_{10}H_{18}O_3$ which Butenandt and Rembold[4] subsequently identified as 10-hydroxydec-2-enoic acid. The presence of the $\alpha\beta$-unsaturated acid moiety has been confirmed chemically[5] and the *trans*-configuration of the olefinic linkage revealed by proton magnetic resonance spectroscopy.[6] Small amounts of sebacic acid and dec-2-en-1,10-dioic acid are also present in royal jelly[7,8] together with a dihydroxydecanoic acid[8]. Since the work described in this paper was essentially complete,[9] syntheses of both the *cis* and *trans*-forms of 10-hydroxydec-2-enoic acid have been described.[10,11] We now record an independent chemical proof of structure of the royal jelly acid together with the results of the proton magnetic resonance spectroscopic studies.

Both methyl 10-methoxy-*cis*- and *trans*-dec-2-enoate were synthesized using an acetylenic route as follows. Heptane-1,7-diol was prepared by sequential application of periodate oxidation and borohydride reduction to cycloheptane-1,7-diol or by reduction of diethyl pimelate with lithium aluminium hydride; it gave[12] a crystalline di-*p*-phenylazobenzoate. The diol was converted[13] to 7-chloroheptan-1-ol (further

* Preliminary reports of part of this work have been published; see Refs. 5 and 6.

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characterized as the *p*-phenylazobenzoate) using cuprous chloride and conc hydrochloric acid and thence, after methylation, to the unstable 1-iodo-7-methoxyheptane by treatment with sodium iodide in acetone. The iodo-compound reacted readily with sodium acetylide in liquid ammonia affording 9-methoxynon-1-yne. The latter compound was converted to the Grignard derivative which reacted with carbon dioxide to give 10-methoxydec-2-ynoic acid characterized as the *S*-benzylthiouronium salt. In preliminary experiments 7-chloroheptan-1-ol was tetra-hydropyranylated and the product converted by treatment with sodium iodide in acetone to the unstable 1-iodo-7-tetrahydropyran-2-yloxyheptane. However, the iodo-compound failed to react with sodium acetylide in liquid ammonia. The reaction does proceed in dimethylformamide.[10]

Reduction of methyl 10-methoxydec-2-ynoate with lithium aluminium hydride gave 10-methoxy-*trans*-dec-2-en-1-ol characterized as the *p*-phenylazobenzoate. The same alcohol was produced by converting naturally occurring 10-hydroxydec-2-enoic acid to the methyl ether methyl ester derivative and reducing with lithium aluminium hydride. Thus, the configuration of the olefinic linkage in the naturally occurring acid is confirmed as *trans*. Oxidation of 10-methoxy-*trans*-dec-2-en-1-ol to the aldehyde with activated manganese dioxide[14] and thence to the acid with silver oxide gave, after esterification, methyl 10-methoxy-*trans*-dec-2-enoate identical with the methyl ether methyl ester of naturally occurring 10-hydroxydec-2-enoic acid. Attempts to demethylate 10-methoxy-*trans*-dec-2-en-1-ol and methyl 10-methoxy-*trans*-dec-2-enoate with boron trichloride[15] gave unidentified products. A synthetic route based on a malonic ester condensation was used by Sir Robert Robinson *et al.*,[11] to obtain 10-hydroxy-*trans*-dec-2-enoic acid.

Hydrogenation of 10-methoxydec-2-ynoic acid over Lindlar's catalyst[16] and esterification of the product gave methyl 10-methoxy-*cis*-dec-2-enoate. An acetylenic route was used by Sir Robert Robinson *et al.*,[10] to synthesize 10-hydroxy-*cis*-dec-2-enoic acid.

For the purpose of comparison of proton magnetic resonance spectra the hitherto unknown model compounds methyl *cis*- and *trans*-undec-2-enoate were also synthesized.

The most characteristic bands in the nuclear magnetic resonance spectra of these compounds were those arising from the system $-\underset{4}{CH_2}-\underset{3}{CH}=\underset{2}{CH}-$. The olefinic absorptions in the spectra of methyl *cis*-undec-2-enoate and methyl 10-methoxy-*cis*-dec-2-enoate corresponded to the AB region of an ABX_2 spin system and were analyzed[17] as such. The X_2-regions were modified by further coupling with adjacent methylene groups. The analogous absorptions in the spectra of the *trans* isomers and of the methyl ester of the natural hydroxy acid were analysed as first order systems. The appropriate parameters are listed in the Table, and show the close relation between the natural material and the two *trans*-models. The data in the Table serve to confirm the stereochemical assignments made above. Thus the observed coupling constants between the olefinic protons are in good agreement with those reported for other

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cis- and *trans*-disubstituted olefins.[18] The τ-values of the β-olefinic protons and of the allylic protons also exhibit typical shifts to lower fields in those isomers in which they are *cis* to the carbomethoxy group.[19]

TABLE 1. COUPLING CONSTANTS (C/S) AND CHEMICAL SHIFTS

	J_{23}	J_{34}	J_{24}	τ_2	τ_3	τ_4
10-Hydroxydec-2-enoate *trans*	15·8	6·9	±1·1	4·18	3·10	7·80
10-Methoxydec-2-enoate *trans*	15·7	7·0	±1·0	4·19	3·12	7·82
Undec-2-enoate *trans*	15·7	6·9	±1·1	4·20	3·12	7·85
10-Methoxydec-2-enoate *cis*	11·4	7·5	−1·2	4·20	3·87	7·35
Undec-2-enoate *cis*	11·3	7·6	−1·3	4·22	3·85	7·32

Although 10-hydroxy-*trans*-dec-2-enoic acid is present in royal jelly in large amount (ca., 70 per cent of ether-soluble material and ca. 15 per cent of total dry weight) and is a secretion of the mandibular glands[6] (not the hypopharyngeal glands as previously supposed) its biological function remains obscure. The acid is only weakly active against bacteria and fungi.[6] Townsend *et al.*,[20] have shown that, on simultaneous injection of 10-hydroxy-*trans*-dec-2-enoic acid and tumour cells, the development of transplantable mouse leukaemia and ascitic tumours is completely suppressed although the acid has no effect on established tumours.

EXPERIMENTAL

Isolation of 10-hydroxy-trans-dec-2-enoic acid from royal jelly. Freeze-dried royal jelly (20·4 g, anhydrous material) was continuously extracted with ether during 18 hr; the ether soluble fraction (3·9 g) comprised 19% of the royal jelly. The ethereal solution was extracted with 0·5 N-sodium carbonate. The aqueous solution was acidified, extracted with ether and the ethereal solution was dried ($MgSO_4$) and concentrated to yield crude fatty acid (2·49 g, 71%). Recrystallization of the crude product from ether at ca. −40° gave 10-hydroxy-*trans*-dec-2-enoic acid (1·13 g) m.p. 52–53°, $[\alpha]_D$ ±0° (*c* 4·5 in dioxan, 1 dm tube). (Found: C, 64·5; H, 9·6; Equiv. wt. 187·9. Calc. for $C_{10}H_{18}O_3$: C, 64·5; H, 9·7%; Equiv. wt. 186).

Heptane-1,7-diol. Diethyl pimelate (65 g) was added to a stirred suspension of lithium aluminium hydride (17 g) in ether (500 ml) at a rate sufficient to maintain gentle boiling. After a further 10 min, water (100 ml) was added dropwise followed by 10% sulphuric acid (900 ml). The solution was extracted continuously with ether overnight, the extract was dried ($MgSO_4$), concentrated and the residue distilled to yield the diol (39 g, 97%) as a colourless syrup, b.p. 156°/18 mm, n_D^{19} 1·4552. The diol gave[12] a *di-p-phenylazobenzoate* m.p. 146–147° (Found: C, 71·6; H, 6·5. $C_{33}H_{32}N_4O_4$ requires: C, 72·2; H, 5·9%).

1-Chloro-7-methoxyheptane. 7-Chloroheptan-1-ol (b.p. 125°/25 mm, n_D^{20} 1·4559) prepared[13] from heptane-1,7-diol gave[12] a *p*-phenylazobenzoate m.p. 75° (Found: C, 67·2; H, 6·35. $C_{20}H_{24}ClN_2O_2$ requires: C, 66·8; H, 6·7%).

Silver oxide (56 g) was added to a stirred solution of 7-chloroheptan-1-ol (24 g) in methyl iodide (69 g). When the exothermic reaction had subsided the mixture was boiled under reflux for 1 hr. Concentration of the cooled, filtered solution and distillation of the residue gave *1-chloro-7-methoxyheptane* (14·49 g, 55%) b.p. 104°/15 mm, n_D^{20} 1·4425 (Found: C, 58·5; H, 10·2. $C_8H_{17}ClO$ requires: C, 58·4; H, 10·3%).

9-Methoxynon-1-yne. A solution of 1-chloro-7-methoxyheptane (9 g) and sodium iodide (10·5 g) in acetone (ca., 70 ml) was boiled under reflux for 18 hr; sodium chloride was precipitated. The filtered solution was evaporated, excess of water was added to the residue and the oily layer was

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separated and dried ($CaCl_2$). Free iodine was removed by shaking with mercury and the product was distilled to yield 1-iodo-7-methoxyheptane (9 g, 64%), b.p. 53°/0·3 mm, n_D^{19} 1·4822. The iodo-compound was very unstable and a satisfactory analysis was not obtained; it was used immediately for the next reaction.

A solution of the iodo-compound (15·2 g) in dry ether (200 ml) was added slowly to a stirred suspension of sodium acetylide, prepared from sodium (4 g) and liquid ammonia (500 ml), and through which acetylene was bubbled at ca., 50 ml/min. After a further 3 hr the gas flow was stopped and the mixture was then allowed to evaporate. Water was added cautiously to the residue and the mixture was extracted with ether. The dried ($MgSO_4$) extract was concentrated and the residue distilled to yield 9-*methoxynon*-1-*yne* (6·2 g, 68%), b.p. 92°/10 mm, n_D^{20} 1·4372 (Found: C, 77·2; H, 11·7. $C_{10}H_{18}O$ requires: C, 77·8; H, 11·8%).

10-*Methoxydec*-2-*ynoic acid*. Ethyl magnesium bromide prepared from magnesium (0·9 g), ethyl bromide (7 g) and ether (10 ml) was treated in an oxygen free atmosphere with a solution of 9-methoxy-1-yne (5·4 g) in ether (10 ml) and the mixture was then boiled under reflux for 3 hr. The cooled (0°) mixture was then treated with an excess of solid carbon dioxide in an autoclave overnight at room temp. The organo-metallic complex was decomposed with dil sulphuric acid and the solution was extracted with ether. The extract was washed with water, then extracted with aqueous sodium hydrogen carbonate. The aqueous extract was acidified and extracted with ether, the extract was dried ($MgSO_4$), concentrated and the residue distilled giving 10-*methoxydec*-2-*ynoic acid* (2·82 g, 41%), b.p. 137°/0·2 mm, n_D^{22} 1·4700 (Found: C, 66·8; H, 9·3. $C_{11}H_{18}O_3$ requires: C, 66·6; H, 9·2%). The acid gave an S-*benzylthiouronium salt* m.p. 170–171° (Found: C, 62·7; H, 7·95; N, 7·6. $C_{19}H_{28}N_2O_3S$ requires: C, 62·6; H, 7·7; N, 7·7%).

Concentration of the ether solution remaining after the sodium hydrogen carbonate extraction gave unchanged 9-methoxynon-1-yne (2·16 g, 40%).

Using essentially the above method dec-1-yne was converted to *undec*-2-*ynoic acid* b.p. 103°/0·3 mm, n_D^{20} 1·4612 (Found: C, 72·8; H, 10·0. $C_{11}H_{20}O_2$ requires: C, 72·5; H, 10·0%) characterized as the S-*benzylthiouronium salt* m.p. 174–175° (Found: C, 65·4; H, 8·0. $C_{19}H_{28}N_2O_2S$ requires: C, 65·5; H, 8·1%).

Methyl 10-*methoxy*-cis-*dec*-2-*enoate*. 10-Methoxydec-2-ynoic acid (396 mg) was added to a suspension of Lindlar's catalyst[16] (75 mg) in ethyl acetate (10 ml) and the mixture was shaken in an atmosphere of hydrogen at a slight overpressure until absorption of gas (ca., 5 ml, 1·07 moles) was complete. The suspension was filtered, the filtrate was concentrated and the residue (350 mg) treated with an excess of freshly prepared diazomethane in ether (10 ml). Concentration of the solution and distillation of the residue gave *methyl* 10-*methoxy*-cis-*dec*-2-*enoate* (232 mg, 61·9%) b.p. 85°/0·25 mm, n_D^{20} 1·4613 (Found: C, 67·2; H, 10·4. $C_{12}H_{22}O_3$ requires: C, 67·25; H, 10·35%).

Reduction of undec-2-ynoic acid essentially as described above gave cis-*undec*-2-*enoic acid* n_D^{18} 1·4516 (Found: C, 71·9; H, 10·6. $C_{11}H_{20}O_2$ requires: C, 71·7; H, 10·9%) which with diazomethane yielded a *methyl ester* n_D^{18} 1·4482 (Found: C, 72·8; H, 11·1. $C_{12}H_{22}O_2$ requires: C, 72·7; H, 11·2%).

10-*Methoxy*-trans-*dec*-2-*en*-1-*ol*. Esterification of 10-methoxydec-2-ynoic acid with diazomethane gave the methyl ester b.p. 133°/18 mm, $n_D^{16·5}$ 1·4585.

A solution of the ester (1 g) in ether (1·4 ml) was added slowly to a stirred suspension of lithium aluminium hydride (1 g) in ether (60 ml) and the mixture was boiled under reflux for 4·5 hr. After addition of water and dil sulphuric acid in the usual manner the mixture was continuously extracted overnight with ether. Concentration of the dried ($MgSO_4$) extract and distillation of the residue gave 10-*methoxy*-trans-*dec*-2-*en*-1-*ol* (0·45 g, 53%) b.p. 102°/0·6 mm, $n_D^{21·5}$ 1,4500 (Found: C, 70·6; H, 12·2. $C_{11}H_{22}O_2$ requires: C, 70·9; H, 11·9%) characterized as the p-*phenylazobenzoate* m.p. 54-55° (Found: C, 73·0; H, 7·7; N, 6·9. $C_{24}H_{28}N_2O_3$ requires: C, 73·4; H, 7·2; N, 7·1%).

Reduction of methyl 10-methoxydec-2-enoate (1·8 g), obtained from the royal jelly acid as described below, with lithium aluminium hydride by essentially the above method, gave an alcohol (1·47 g, 93%) the I.R. spectrum (liquid film) of which was indistinguishable from that of the synthetic 10-methoxy-*trans*-dec-2-en-1-ol.

trans-*Undec*-2-*en*-1-*ol*. A mixture of undec-2-ynoic acid (4·7 g), ethanol (6 ml), toluene (3 ml) and conc sulphuric acid (2–3 drops) was distilled onto potassium carbonate (2·5 g) until the distillate temperature reached 78°. The distillate was returned to the still and the process was repeated. The residue was finally distilled to yield *ethyl undec*-2-*ynoate* (4·58 g, 87%) b.p. 128°/10 mm, n_D^{18} 1·4520 (Found: C, 74·0; H, 10·3. $C_{13}H_{22}O_2$ requires: C, 74·2; H, 10·5%).

Reduction of the ester (3·95 g) with lithium aluminium hydride as described above gave trans-*undec-2-en-1-ol* (2·96 g, 92%) b.p. 71°/0·3 mm, n_D^{18} 1·4539 (Found: C, 77·6; H, 12·8. $C_{11}H_{22}O$ requires: C, 77·45; H, 12·9%).

Methyl 10-*methoxy*-trans-*dec-2-enoate*. A solution of 10-methoxy-*trans*-dec-2-en-1-ol (0·4 g) in light petroleum (b.p. 40–60°) was added to a stirred suspension of activated manganese dioxide[14] (5 g) in the same solvent (20 ml). After 2 hr the mixture was filtered, the residue was exhaustively extracted with hot light petroleum and the combined extracts were concentrated yielding crude 10-methoxy-*trans*-dec-2-enal (0·25 g, 62%). The instability of this compound prevented purification.

To a mixture of silver oxide (1·25 g), crude 10-methoxy-*trans*-dec-2-enal (0·25 g) ethanol (0·25 ml) and water (1 ml) was added a solution of sodium hydroxide (0·2 g) in water (0·4 ml). The suspension was shaken overnight then filtered and the insoluble material was washed with water. The combined filtrate and washings were extracted with ether, then acidified and again extracted. The dried ($MgSO_4$) second ether extract was concentrated and the acidic product was esterified with ethereal diazomethane. Concentration of the solution and distillation of the residue gave methyl 10-*methoxy*-trans-*dec-2-enoate* (0·12 g, 41·3%) b.p. 72°/0·1 mm, n_D^{21} 1·4517 (Found: C, 67·4; H, 10·3. $C_{12}H_{22}O_3$ requires; C, 67·25; H, 10·35%).

Methylation of 10-hydroxydec-2-enoic acid (1·72 g), isolated from royal jelly, with silver oxide (6·96 g) and methyl iodide (8·6 g) using the method described above for 7-chloro-1-methoxyheptane gave a product (1·7 g) the I.R. spectrum (liquid film) of which was indistinguishable from that for synthetic methyl 10-methoxy-*trans*-dec-2-enoate.

Oxidation of *trans*-undec-2-en-1-ol by essentially the above method and esterification of the product gave *methyl* trans-*undec-2-enoate* b.p. 79°/0·15 mm, $n_D^{20·5}$ 1·4465 (Found: C, 72·7; H, 11·2. $C_{12}H_{22}O_2$ requires: C, 72·7; H, 11·2%).

7-*Iodoheptan*-1-*ol*. Treatment of 7-chloroheptan-1-ol (15 g) with a 15% solution of sodium iodide in acetone (75 ml) as described above for 1-chloro-7-methoxyheptane gave 7-*iodoheptan*-1-*ol* (17·6 g, 73%) b.p. 77°/0·2 mm, n_D^{17} 1·5174 (Found: C, 34·4; H, 6·4. $C_7H_{15}IO$ requires: C, 34·7; H, 6·25%).

1-*Chloro*-7-*tetrahydropyran*-2-*yloxyheptane*. To a solution of 7-chloroheptan-1-ol (15 g) in freshly distilled dihydropyran (8·5 g) was added conc hydrochloric acid (1 drop) and the mixture was stored overnight. Ether (20 ml) was added and the solution was shaken with aqueous 10% sodium hydroxide (50 ml). The dried ($MgSO_4$) ethereal solution was concentrated and the residue distilled to give the *product* (14 g, 60%) b.p. 168–169°/22 mm, n_D^{20} 1·4613 (Found: C, 61·9; H, 9·8; Cl, 15·3. $C_{12}H_{23}ClO$ requires: C, 61·4; H, 9·8; Cl, 15·1%).

Nuclear magnetic resonance spectra. These were determined with 7% solutions in carbon tetrachloride with a Varian Associates 4300 spectrometer operating at 56·4 Mc/sec. The spectra were calibrated by the side band technique with a Muirhead-Wigan D-695-A audiofrequency oscillator.

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