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# The Biological Activity and Synthesis of Royal Jelly Acid*

*Sir Robert Robinson*

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In the issue of *Nature* that appeared on May 2nd, 1959 (**183**, 1270) G. F. Townsend, J. F. Morgan, and B. Hazlett published an important paper showing that the Royal Jelly of Bees (the food of queen larvae, produced in the mandibular glands of worker bees) inhibited the development of transplantable leukaemia and three varieties of ascitic tumours in mice. The main fatty acid of Royal Jelly was isolated by Townsend and Lucas in 1940[1] and later shown by Butenandt and Rembold[2] to be 10-hydroxydec-2-enoic acid, $HOCH_2(CH_2)_6\,CH{:}CH\cdot CO_2H$. There is about 3% of this substance in Royal Jelly and its biological activity in the above-mentioned sense was found to be from 20 to 100 times as great. Hence this fatty acid is the active constituent.

With my colleagues G. I. Fray and R. H. Jaeger the synthesis of the acid was effected.[3] The configuration of the acid was unknown; it proved to be *trans* (infra-red spectrum) and the m. p. was raised from 54—56° to 64—65°. The more practicable of the syntheses is also the simplest.

When castor oil is heated with alkaline solutions it affords 10-hydroxydecanoic acid[4] in moderate yield, along with other products. The hydroxyl group is then acetylated.[5,6] Alternatively, undecylenic acid is reduced to undecenyl alcohol which is acetylated and oxidised to 10-acetoxydecanoic acid. The corresponding acid chloride is brominated, the product hydrolysed to the acid, $AcOCH_2(CH_2)_7CHBr\cdot CO_2H$, which is successively treated with sodium iodide in ethanol, and aqueous ethanolic sodium hydroxide. The product is the *trans*-R. J. acid identical with the natural product kindly supplied by Dr. Rembold of the Max-Planck Institute for Biochemistry, München, and also by Dr. W. H. Brown of the Ontario Agricultural College, Guelph, Canada.

The two specimens were purified by crystallisation from ether-light petroleum (b. p. 40—60°) and the mixed melting points with the synthetical specimen were undepressed. The infra-red absorption curves were also identical. A second synthesis was from 8-acetoxyoctanal, $AcOCH_2(CH_2)_5\cdot CHO$, b. p. 81°/0.3 mm., by condensation at 90° with malonic acid in pyridine containing a little piperidine (Doebner synthesis). The resulting acetoxy-acid was hydrolysed with formation of the *trans*-R. J. acid identical with that previously prepared.

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**8-Acetoxyoctanal** was made from 6-chlorohexanol[7] by condensation with sodio diethylmalonate, hydrolysis, decarboxylation, and acetylation to 8-acetoxyoctanoic acid, followed by Rosenmund reduction of the acid chloride.

$$HO(CH_2)_6Cl \longrightarrow HO(CH_2)_6 \cdot CH(CO_2Et)_2 \longrightarrow HO(CH_2)_7CO_2H$$
$$\longrightarrow AcO(CH_2)_7COCl \longrightarrow AcO(CH_2)_7CHO$$

Alternatively 8-acetoxy-1-bromo-octane[8] was converted into 8-acetoxyoctyl toluene-*p*-sulphonate and the latter oxidised by dimethyl sulphoxide.[9] The *trans*-configuration of R. J. acid has also been deduced from the results of a study of the nuclear magnetic resonance spectrum of the methyl ester obtained by the action of diazo-methane on the acid (from natural sources) dissolved in carbon tetrachloride.[10]

Dr. W. H. Brown (private communication) has also synthesised R. J. acid by a modification of our second method and Professor R. A. Raphael (private communication) has made it by the following interesting route:

$$AcO(CH_2)_8 \cdot C{\equiv}CH \xrightarrow[KOBr]{} AcO(CH_2)_8CO \cdot CHBr_2 \longrightarrow$$
$$\xrightarrow{\text{Favorski rearrangement}} HO(CH_2)_7\,CH{=}CH \cdot CO_2H.$$

Our first method is certainly the best from a preparative point of view.

The *cis*-10-hydroxydec-2-enoic acid[12] has also been synthesised[11] starting with pimelic acid which was esterified and reduced by $LiAlH_4$ to heptan-1,7-diol which afforded the chlorohydrin, $HO(CH_2)_7Cl$, on treatment with hydrochloric acid and cuprous chloride[13]. The tetrahydropyranyl ether of this substance was treated with sodium iodide in acetone and then with sodium acetylide in dimethylformamide, yielding 9 - 2' - tetrahydropyranyloxynon-1-yne, b. p. 90—91°/0.3 mm.

This acetylene reacted with ethylmagnesium bromide[14] followed by solid carbon dioxide to give a tetrahydropyranyloxy-acid which was hydrolysed to 10-hydroxydec-2-ynoic acid, $HO(CH_2)_7 \cdot C \vdots C \cdot CO_2H$, m. p. 72—73°. Hydrogenation in presence of Lindlar's catalyst[15] gave an almost theoretical yield of *cis*-10-hydroxydec-2-enoic acid. The *cis* C=C group is indicated by absorption bands at 6·15 and 12·3 μ. The UV maximum was at 210 mμ, ε 12,450 as against 211 mμ, ε 12000 for the *trans*-isomeride.

According to Brown and Freure[16] irradiation of 10-hydroxydec-2-enoic acid (m. p. 62°) in acetone solution gave a product, m. p. 43—46°, which after 3 weeks had m. p. 44—54°. This was interpreted as a conversion of the *trans*- to the *cis*-isomeride on irradiation, but it is evident that, if that explanation is correct, the change was far from complete. These authors (*loc. cit.*) have isolated sebacic acid and 2-decendioic acid from Royal Jelly.

Reverting to the biological properties of R. J. acid, it must be emphasised that the Canadian group obtained dramatic results when the cells to be used as inoculum were treated with a sufficient concentration of R. J. acid, at a pH on the acid side, prior to inoculation. They have since employed the methyl ester (private communication).

There is as yet no evidence of a control of the tumour when it is already implanted. In considering a possible mechanism of the effects from a chemical point of view, the author has had in mind the rather innocuous nature of R. J. acid and has formed the idea that it may have a physical or pseudo-physical effect through the formation of a polyester, which could be relatively simple or macromolecular:

$$n\ [HO(CH_2)_7CH{=}CH\cdot CO_2H] \longrightarrow$$
$$HO\cdot(CH_2)_7\cdot CH{=}CH\cdot CO - [O(CH_2)_7\cdot CO]_{n-2}\ O(CH_2)_7CH{=}CH\cdot CO_2H.$$

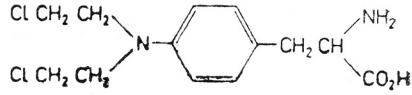
Such molecules could adhere or combine with the cell surface and the $\alpha\beta$-double bonds supply further possibilities for linking and cross-linking. In this way the pores on the surfaces might be closed and the cell deprived of nutriment. Naturally a similar conception could apply to other parts of the make-up of the cells but the surface seems the most probable. It is most important to examine this possibility closely as, if the phenomenon is a real one, it may even suggest methods for practical treatment.

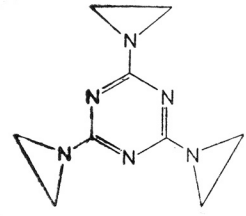
Something quite similar has been found in the anti-tuberculous effects of certain surface-active polyoxyethylene ethers.[17] None of these substances had any direct anti-bacterial action and it was postulated that they act indirectly on the larger structures (monocytes) in which the bacilli proliferate. A very interesting point was that by increasing the length of the polyethylene glycol ether chain, the lipophilic/hydrophilic ratio could be progressively altered and, in the case of an active series, as the ratio decreased activity passed from anti-tuberculous ——→ inactive ——→ protuberculous.

E. J. Ambrose and his collaborators have studied the specific properties of malignant cells and especially the reactions of polyelectrolytes with the surfaces of normal and tumour cells.[18] Their summary runs as follows.

> »Previous evidence has indicated that the surface of cancer cells differs from that of normal cells and that these cells carry a higher negative electrical charge than those from which they are derived. Studies have therefore been made of the adsorption of positively charged polymers by these cells as a possible means of reducing the surface charge. One polymer (polyethylene imine) has been found to show some specificity towards tumour cells.«

Finally, without making any particular deduction from it, the fact is that all the partly successful therapeutic anti-cancer agents so far encountered, are bis- or poly-functional. Examples are:




$MeOSO_2(CH_2)_4SO_2OMe$

Professor Townsend and his colleagues have meanwhile announced that dibasic acids, for example sebacic acid, are able to inhibit the development of tumours in animals when pre-mixed with inoculum. The formation of polyesters or polyamides or amide esters from such dibasic acids and compounds containing more than one hydroxyl or amino group is naturally quite possible and the lack of specifity in the process favours the idea of a physical mechanism as already presented in this communication.

REFERENCES

1. G. F. Townsend and C. C. Lucas, *Biochem. J.* **34** (1940) 1155.
2. A. Butenandt and R. Rembold, *Z. physiol. Chem.* **308** (1957) 284.
3. *Tetrahedron Letters* **4** (1960) 15; *U. K. Patent Application* No. 25692/59 of 27. VII. 1959.
4. *U. K. Patent* No. 675, 434.
5. A. Grün and T. Wirth, *Ber.* **55** (1922) 2206.
6. P. Chuit, F. Boelsing, J. Hausser, and G. Malet, *Helv. Chim. Acta* **9** (1926) 1074.
7. W. R. Coleman and W. G. Bywater, *J. Am. Chem. Soc.* **66** (1944) 1821.
8. F. L. M. Pattison, W. C. Howell, A. J. McNamara, J. C. Schneider, and J. F. Walker, *J. Org. Chem.* **21** (1956) 739.
9. N. Kornblum, W. J. Jones, and G. J. Andersen, *J. Am. Chem. Soc.* **81** (1959) 4113.
10. S. A. Barker, A. B. Foster, D. C. Lamb, and L. M. Jackman, *Nature* **184** (1959) 634.
11. G. I. Fray, E. D. Morgan, and R. Robinson, *Tetrahedron Letters* (in the press).
12. *U. K. Patent Application* No. 8576/60.
13. Compare W. R. Coleman and W. G. Bywater, *J. Am. Chem. Soc.* **66** (1944) 1821.
14. Compare J. H. Wotis, C. A. Hollingsworth, and R. E. Dessy, *J. Am. Chem. Soc.* **78** (1956) 1221.
15. H. Lindlar, *Helv. Chim. Acta* **35** (1952) 446.
16. W. H. Brown and R. J. Freure, *Canadian J. of Chem.* **37** (1959) 2046.
17. J. W. Cornforth, P. D'arcy Hart, G. A. Nicholls, R. J. W. Rees, and J. A. Stock, *Brit. J. of Pharm. and Chemotherapy* **10** (1955) No. 1, 73.
18. E. J. Ambrose, D. M. Easty, and B. C. T. Jones, *Brit. J. of Cancer* XII (1958) 439.

## IZVOD

**Biološka aktivnost i sinteza kiseline izolirane iz matične mliječi**

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Dokazano je da aktivni konstituent matične mliječi, 10-hidroksi-2-decenska kiselina, ima *trans* konfiguraciju. Opisana je sinteza toga spoja iz undecilenske kiseline i alternativna sinteza iz 8-acetoksioktanala kao polaznog produkta. Nisu navedeni eksperimentalni podaci za ove sinteze. Razmatra se mogući mehanizam biološkog djelovanja s kemijske tačke gledišta.

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