Stereochemistry and vitalism

Keith Manchester*

IN 1898, FRANCIS JAPP, PROFESSOR OF chemistry at the University of Aberdeen in Scotland, delivered a presidential address entitled 'Stereochemistry and vitalism' to the Chemistry section of the British Association for the Advancement of Science.[1] In it, he pointed out the importance of the discoveries of stereochemistry 'because it furnishes a reply to the most fundamental question that physiology can propose to itself – namely, whether the phenomena of life are wholly explicable in terms of chemistry and physics; or whether, on the contrary, there are certain residual phenomena, inexplicable by such means, pointing to the existence of a directive force which enters upon the scene of life itself, and which, whilst in no way violating the laws of the kinetics of atoms, determines the course of their operation within the living organism.'

Japp was referring to the existence of the optically active single chiral[a] forms of the products of nature. The organic chemist, on the other hand, unless he employs an already existing enantiomorph or some other facet of life—for example, Louis Pasteur consciously separating the right- and left-faceted crystals of sodium ammonium *para*tartrate—can produce but the racemic (mixed) form. 'Living matter', Japp pointed out, 'is constantly performing a certain geometrical feat which dead matter … is incapable—not even conceivably capable—of performing.'

Influence of Japp's lecture

Probably Japp's lecture would have retained less interest if it had not been used by the crime writer Dorothy L. Sayers in her novel *The Documents in the Case*,[2] published in 1930. In it, a keen middle-aged amateur mycologist, George Harrison, is mysteriously found dead after eating a stew supposedly made from the edible mushroom warty caps (*Amanita rubescens*: Fig. 1) he had intimated his intention of collecting. Forensic examination suggested poisoning by muscarine, an alkaloid found in fly agaric (*Amanita muscaria*: Fig. 2).[b] Did Harrison gather fly agaric by mistake, a possibility stoutly denied by his son, or was there another explanation—suicide or murder?[3]

Fig. 1. *Amanita rubescens.*

From the import of Japp's lecture, the alkaloid muscarine from fly agaric would be expected to be optically active, but careful analysis of the muscarine found in vomit from Harrison and in the stew showed it to be optically inactive, that is, a racemic mixture. This suggested that it had been synthesized in a laboratory and added to the stew by the emerging murderer, who proved to be the lover of Harrison's beautiful second wife, 'tripped up' in the event, as Sayers said, 'by a miserable asymmetric molecule.'

Chemistry of muscarine

Muscarine (Latin *musca*, a fly) was original isolated and named by Erich Harnack and Oswald Schmiedeberg in 1875.[4] Their proposed structure **1** (see structural formulae on the next page) was closely related to that of choline, with -$CH(OH)_2$ replacing -$CH_2(OH)$. Muscarine, they suggested, could be generated from choline, as described in Sayers' novel, by treatment of choline with nitric acid (Fig. 3). The pharmacology was unconvincing, however, and in 1914 Arthur Ewins[5] showed that treatment of choline with nitric acid produced choline nitrous ester **2**, not the putative muscarine.

Fig. 2. *Amanita muscaria.*

Harnack and Schmiedeberg's structure **1** does not possess a chiral centre and, up to the time of Sayers' novel, there was no reason to suppose that natural muscarine was optically active. Indeed, following publication of the novel, Sayers received[6] 'the inevitable letter from a very polite professor of chemistry' pointing out her error.[c]

So why had Sayers thought that muscarine from fly agaric would be optically active? Sayers was a daughter of the church and a graduate in modern languages and had minimal knowledge of chemistry. It was a Dr Barton[d], who was to become her co-author, who had suggested to her in 1928 a possible plot for a crime story in which a murder might be committed by the addition of the synthetic form of a poisonous natural product, for instance an alkaloid, to a supposedly harmless food, such as a stew, in the, as would be shown, mistaken belief that there could be no way of recognizing that the detected poison had come other than from a natural source. Barton must

Fig. 3. Dr Barton demonstrates to Sayers the laboratory preparation of muscarine from choline and nitric acid. From ref. 25.

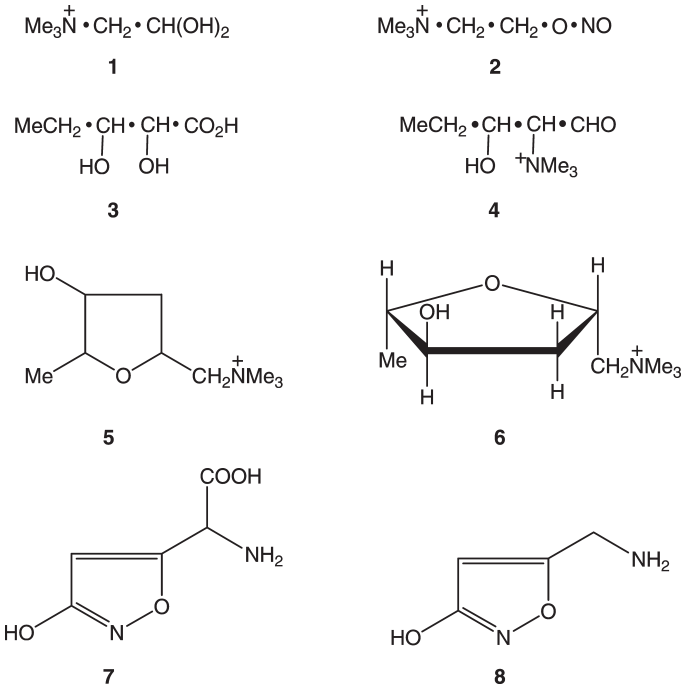
[a]Chiral – from the Greek for a hand. Our two hands constitute non-superposable mirror images of each other. Chiral compounds arise in organic chemistry when the four valencies of a carbon atom are attached to four different groups, resulting in two mirror image non-superposable molecular orientations, one of which in solution will rotate the plane of polarized light to the right (*d*- or +), the other to the left (*l*- or –). The two forms are enantiomorphs of each other and are often designated by a convention introduced by Emil Fischer as D- and L-, where the capital letters relate to structural forms and do not directly relate to *d*- and *l*- , which are purely empirical. Chemists today have replaced Fischer's D- and L- with an *R* (Latin *rectus*, right) and *S* (Latin *sinister*, left) convention.

*School of Molecular and Cell Biology, University of the Witwatersrand, Private Bag 3, WITS 2050, South Africa. E-mail: kmanches@biology.wits.ac.za

[b]Fly agaric is so called because country people formerly used the juice of the stewed mushroom as a fly repellent.

[c]According to Brabazon,[8] the professor who wrote to Sayers suggested that muscarine was not optically active because it was not a protein, but this seems unlikely.

[d]Dr Eustace Robert Barton (1868–1943) already had experience of writing medical mystery stories using the pseudonym of Robert Eustace. *The Documents in the Case* is the only one of Sayers' major novels written jointly with another author and the only one in which the services of Lord Peter Wimsey are not required.



Structural formulae of compounds discussed in text.

have read either Japp's lecture to the British Association or, more likely, the very full report accorded to it in *The Times* newspaper,[7] since as one of Sayers' characters remarks, 'As Professor Japp said, as long ago as 1898,' and then proceeded with verbatim extracts from the lecture.

Barton was apparently much put out by the letter from the 'very polite professor of chemistry',[8] but relief was soon at hand. Another professor of chemistry, J.T. Hewitt,[9] in 1932, in reviewing the second edition of Schmidt's *Textbook of Organic Chemistry*, commented how the structure **1** given for muscarine was unlikely to be correct, since the Hofmann degradation of muscarine base with silver oxide described by Kögl *et al.*[10] in 1931 produced an optically active form of dihydroxy-*n*-valeric acid **3**. Hewitt (tongue in cheek?) 'thanked the authors of an ingenious detective novel[2] for calling his attention to the optical activity of muscarine'(!), something which structure **1** would not possess. In the seventh edition of Schmidt's text (1955), it is commented that the constitution of muscarine 'is not yet established with certainty. Possibly it is a basic hydroxy-aldehyde' **4**, that is, as proposed by Kögl *et al.*[10] This edition preceded the work of Eugster and others in the late 1950s, when the structure **5** was clearly established,[11] the final details being resolved by X-ray crystallography.[12] Structure **6** is possibly more accessible to the biochemist or biologist.[e]

Sayers and Barton were in principle correct in believing that synthetic muscarine, if made from simple non-chiral starting materials, would be a racemic mixture and hence not optically active. However, optically active muscarine can be synthesized either if one of the starting components is one of the enantiomers of a naturally occurring chiral compound, e.g. L-chitaric acid,[13] 2-deoxyribose[14] or D-mannitol,[15] or if a chiral compound, e.g. (-)-di-*p*-toluoyltartaric acid,[11] or an enzyme[16] is used to differentiate one of the chiral components at some stage in the preparation. These last two procedures were used by Pasteur to isolate the individual enantiomers of tartaric acid. The structures **5** and **6** [the quaternary trimethylammonium salt of (2S; 3R; 5S)-5-aminomethyltetrahydro-3-hydroxy-2-methylfuran], possess three chiral centres, and exhibit epi and allo and epiallo forms. Natural muscarine is L-(+).

Fig. 4. *Inocybe patouillardii*.

[e]In fact, *A. muscaria* is far from the richest source of muscarine, its toxicity being due more to its content of the hallucinogenic ibotenic acid **7** and its decomposition product muscimol **8**. Members of the *Inocybe* family such as *I. patouillardii* (Fig. 4) contain several hundredfold higher concentrations of muscarine.[24]

Fig. 5. Portrait of Francis Japp (1848–1926): *J. Chem. Soc.* 1008 (1926).

Professor Francis Japp

Francis Robert Japp (Fig. 5) was born in Dundee, Scotland, in 1848. After education at St Andrews University, he worked in Germany first with Robert Bunsen in Heidelberg, then with August Kekulé in Bonn. From 1877 to 1890, when he went to Aberdeen, he worked in London at the Normal School of Science, a precursor institution of Imperial College London (founded in 1907). While there, in 1888, he and a student developed the Japp–Klingemann reaction,[17] basically the formation of hydrazones by coupling of aryldiazonium salts with active methylene compounds in which at least one of the activating groups is acyl or carboxyl, which group usually cleaves during the process. The careful investigation of a large number of such condensation reactions and the elucidation of the constitution of the compounds produced in these reactions or prepared as derivatives from them, not only formed a very important and valuable contribution to synthetic organic chemistry, but also enabled Japp to elucidate the constitution of important compounds prepared by other investigators.

Japp's lecture to the British Association is erudite and logically consistent. It makes no attempt to introduce religious or similar considerations. It is largely a reconsideration of Pasteur's 'Lectures on the molecular asymmetry of natural organic products', delivered in 1860 before the Chemical Society of Paris.[18] In them, Pasteur argued that 'Artificial products have no molecular asymmetry; and I could not point out the existence of any more profound distinction between the

products formed under the influence of life, and all others.' He also refers to 'the molecular asymmetry of natural organic products' as 'the great characteristic which establishes perhaps the only well-marked line of demarcation that can at present be drawn between the chemistry of dead matter and the chemistry of living matter.' As Japp comments,[1] 'Pasteur's point is, that whereas living Nature can make a *single* optically active compound, those laboratory reactions, to which we resort in synthesizing such compounds, always produce, simultaneously, at least *two*, of equal and opposite optical activity; the result being intermolecular compensation and consequent optical inactivity.'

The implications of these views were not shared by all. Emil Fischer, quoted by Japp,[1] in a lecture, 'Syntheses in the sugar group', given to the German Chemical Society in 1890, asks: 'Is the preparation of optically active substances a prerogative of the living organism; is a special cause, a kind of vital force, at work here? I do not think so, and incline rather to the view that it is only the imperfection of our knowledge which imports into the process the appearance of the miraculous.' But, as Japp continues, until the miraculous can be explained, 'the *absolute origins* of the compounds of one sided asymmetry to be found in the living world is a mystery as profound as the absolute origin of life itself. The two phenomena are intimately connected, for … these asymmetric compounds make their appearance with life, and are inseparable from it.'

Modern views

A hundred years on, to what extent has the 'mystery' been dispelled? We now are well aware that the asymmetry of many natural products is a consequence of their synthesis by asymmetric enzymes, whose asymmetry results at least in part from their content of asymmetric amino acids, all of the L-configuration. But Japp would presumably have little difficulty in accepting such advances within the scope of his arguments, even the ability of enzymes such as aconitase to distinguish between the two ends of the symmetrical and achiral citrate.[19] We are, however, little further forward in our understanding of how it arose that only one enantiomorph of the amino acids came to be the sole component of proteins.

Many simple biological compounds including the amino acids, sugars and nucleotide bases seem to form relatively easily as a result of electrical discharges through various mixtures of methane, ammonia, nitrogen, hydrogen and carbon dioxide, but with no specific chirality.[20] In this context it is noteworthy that all *ab initio* calculations of the parity-violating energy differences predict that the naturally occurring left-handed amino acids are indeed more stable than their enantiomers, as are some key D-sugars and right-handed helical DNA.[21] Selective enantio-enrichment of tartaric acids can be made with circularly polarized light (CPL), but left-CPL only serves to destroy the D-tartaric acid whereas the right-CPL destroys the L-tartaric acid.[22] Rikken and Raupach have shown that magnetochiral anisotropy—an effect linking chirality and magnetism—can give rise to an enantiomeric excess in a photochemical reaction driven by unpolarized light in a parallel magnetic field.[23] Such an effect, the authors speculate, may have played a role in the origin of the homochirality of life. Indeed, Pasteur 150 years ago tried unsuccessfully to demonstrate something similar. However, the yields are very small. It seems that over the question of the origin of the homochirality of life, the jury is still out.

1. Japp F.R. (1898). Stereochemistry and vitalism. In *Report of the 68th meeting of the British Association for the Advancement of Science*, pp. 813–828. John Murray, London.
2. *The Documents in the Case*, by Dorothy L. Sayers and Robert Eustace. Ernest Benn, London (1930).
3. Hart H. (1975). Accident, suicide, or murder? A question of stereochemistry. *J. Chem. Educ.* **52**, 444.
4. Harnack E. and Schmiedeberg O. (1875). Fliezenpils-Alkaloide. *Arch. Exp. Path. Pharm.* **4**, 168–194.
5. Ewins A.J. (1914). The constitution of pseudomuscarine ('synthetic muscarine'). *Biochem. J.* **8**, 209–215.
6. Sayers D.L. (1932). Trials and sorrows of a mystery writer. *The Listener* (London) 7, 6.
7. *The Times* (London), 9 September 1898, p. 8.
8. Brabazon J. (1981). *Dorothy L. Sayers*: *The Life of a Courageous Woman.* Victor Gollancz, London.
9. Hewitt J.T. (1932). Review. *The Analyst* **57**, 593–595.
10. Kögl F., Duisberg H. and Erxleben H. (1931). Untersuchungen über Pilzgifte. I. Über das Muscarin. *Ann. der Chemie* **489**, 156–192.
11. Eugster C.H. (1960). The chemistry of muscarine. *Adv. Org. Chem.* **2**, 427–455.
12. Jellinek F. (1957). The structure of muscarine. *Acta Cryst.* **10**, 277–280.
13. Hardegger E. and Lohse F. (1957). Über Muscarin. Synthese und absolute Konfiguration des Muscarins. *Helv. Chim. Acta* **40**, 2383–2389.
14. Pochet S. and Huynh-Dinh T. (1982). Stereospecific synthesis of muscarines and allomuscarines in D and L series. *J. Org. Chem.* **47**, 193–198.
15. Mubarak A.M. and Brown D.M. (1980). A simple, stereospecific synthesis of (+)-muscarine. *Tetrahedron Lett.* **21**, 2453–2454.
16. Whiting J., Au-Young Y-K. and Belleau B. (1972). A convenient synthesis of L(+)-muscarine. *Can. J. Chem.* **50**, 3322–3325.
17. Japp F.R. and Klingemann F. (1888). The constitution of certain so-called 'mixed azo-compounds'. *J. Chem. Soc. Trans.* **53**, 519–544.
18. Pasteur L. (1860). Recherches sur la dissymétrie moléculaire des produits organiques naturels. *Oeuvres* **1**, 314–344.
19. Ogston A.G. (1948). Interpretation of experiments on metabolic processes, using isotopic tracer elements. *Nature* **162**, 963.
20. Miller S.L. (1987). Which organic compounds could have occurred on the prebiotic earth? *Cold Spring Harbor Symp. Quant. Biol.* **52**, 17–27.
21. MacDermott A. (1996). In *Physical Origin of Homochirality in Life,* ed. D.B. Cline, pp. 241–254. American Institute of Physics, Woodbury, NY.
22. Shimizu Y. (1997). Laser-induced enantioenrichment of tartaric acid *via* a multiphoton absorption process. *J. Chem. Soc., Perkin Trans.* **1**, 1275–1278.
23. Rikken G.L.J.A. and Raupach E. (2000). Enantioselective magnetochiral photochemistry. *Nature* **405**, 932–935.
24. Wilkinson S. (1961). The history and chemistry of muscarine. *Chem. Soc. Quart. Rev.* **15**, 153–171.
25. *The Letters of Dorothy L. Sayers. 1899–1936: The Making of a Detective Novelist.* Chosen and edited by Barbara Reynolds with a preface by P.D. James. Hodder & Stoughton, London (1995).