Fascinating Organic Molecules from Nature

4. Hunting with Poisoned Arrows: Story of Curare

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(left) N R Krishnaswamy was initiated into the world of natural products by T R Seshadri at University of Delhi. He has taught at Bangalore University, Calicut University and Sri Sathya Sai Institute of Higher Learning. He has the uncanny ability to present the chemistry of natural products logically and with feeling.

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Wieland–Gumlich aldehyde, curare, d-tubocurarine, d-beeberine, toxiferine-1, deoxytoxiferine, hemitoxiferine.

European explorers of the New World brought back to Europe exotic specimens of plant and animal origin. One of these was curare, the arrow poison used by the natives of the Orinoco and the Amazon river basins mainly to capture birds and animals for food. Three types of curare were known, namely tubocurare, calabash curare and pot curare. The main plant component of tubocurare was identified as the Menispermaceae plant *Chondrodendron tomentosum*, whereas in calabash curare the chief ingredient was the bark of *Strychnos toxifera*. From tubocurare, the quaternary base, d-tubocurarine was isolated. It is a bis-benzylisoquinoline alkaloid. Calabash curare is a complex mixture of several closely related indole alkaloids.

Introduction

Indigenous people in all parts of the world have used locally available plant materials for various purposes and in the process have developed ingenious methods for their utilization in diverse activities including hunting for animal food. European explorers of South America brought back to Europe not only a wealth of information about the life and culture of the natives of these regions but also specimens of exotic materials. One of these was curare, derived from the word *wurari* which means poison in the language spoken by the tribal people of Guyana. There are three types of curare, namely, tubocurare, calabash curare and pot curare. In the first case, hollow bamboo tubes are used for packing the processed plant material whereas hollow gourds and terra cotta pots are used as containers for calabash curare and pot curare respectively.

Sir Walter Raleigh, a multifaceted English nobleman in the courts

of Elizabeth I and James I, was, perhaps, the first European to have observed the use of poisoned arrows by the indigenous people of Guyana. He recorded his observations in a book *The Discoverie of Guiana* published in 1596. However, an authentic description of the preparation of a curare was given two centuries later by Alexander von Humboldt. Being a keen observer and an outstanding raconteur he recorded his discoveries in a series of volumes. With the specimens brought back to Europe, the pharmacological properties of curare were studied in several laboratories. However, the most significant observation was made only in 1935 by the Nobel Laureate (Medicine) Sir Henry H Dale. He found that curare is a skeletal muscle relaxant and acts by blocking the action of the neurotransmitter, acetyl choline. Following this discovery and the observation that curare is not toxic when administered orally it was introduced in anesthesia in the early 1940s.

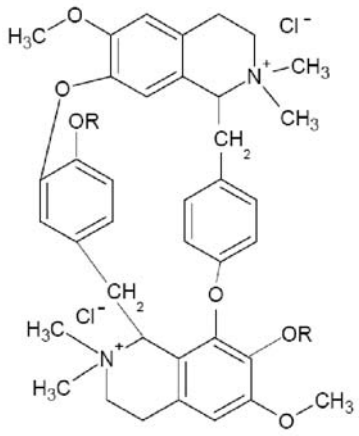
Previous articles:
1. Some Exotic Red Pigments of Plant Origin, Vol.17, No.10, 2012.
2. The Blue of Blue Jeans and Royal Purple, Vol.17, No.11, 2012.
3. Colours in Flight – Pigments from Bird Feathers and Butterfly Wings, Vol.18, No.1, 2013.

Chemistry of d-Tubocurarine

The major toxic principle of tubocurare is d-tubocurarine, a crude sample of which was first isolated by the German chemist, Boehm, in 1897. However, it was only in 1935 that a pure, crystalline form of the compound could be obtained. This was achieved by H King who was working in the laboratories of H H Dale[1]. He isolated the alkaloid from a museum specimen of curare. King also established the structure of this alkaloid exclusively on the basis of classical chemical methods of analysis, degradation and synthetic studies. From a historical viewpoint this piece of research work, indeed, has high instructional value.

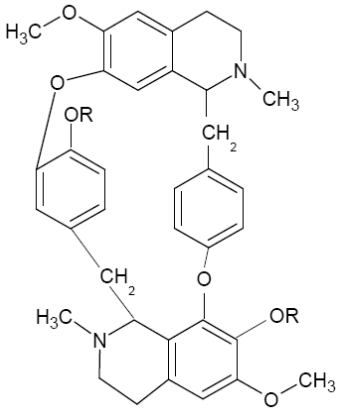
[1] Sir Henry Hallett Dale, OM, FRS, was a pioneer physiologist -pharmacologist. He is best known for his studies on the neurotransmitter, acetyl choline, a work that earned for him the Nobel Prize in Physiology or Medicine in the year 1936, jointly with his life-long friend, Otto Loewi. Dale also unraveled the physiological activities of histamine and made a thorough study of the pharmacological properties of the ergot alkaloids.

King determined the molecular formula of d-tubocurarine as $C_{38}H_{44}N_2O_6Cl_2$. Zeisel estimation showed the presence of two methoxyl groups. The presence of two phenolic hydroxyls was proved by the formation of a di-O-methyl ether by the action of methyl iodide. d-Tubocurarine (**1**) as well as its di-O-methyl ether (**2**) were found to be quaternary ammonium chlorides. Further experiments ultimately proved that (**1**) is the dimethochloride of the alkaloid d-beeberine (**3**).



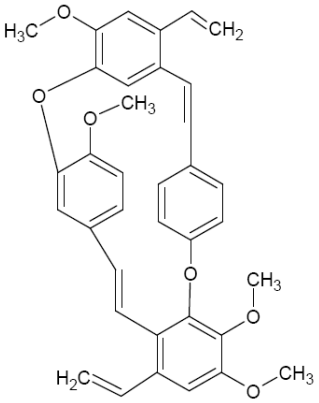
1: R=H

2: R=CH_3



3: R=H

7: R=CH_3



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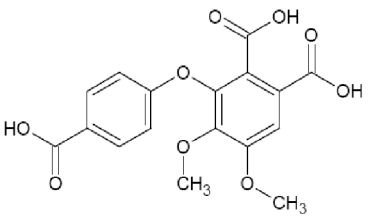
A Hofmann degradation of (**2**) gave a mixture of four methine bases which was further methylated and subjected to a second Hofmann degradation. This resulted in the formation of trimethylamine and a nitrogen-free compound (**4**). On oxidative degradation, (**4**) gave a tricarboxylic acid (**5**) which on fusion with alkali yielded 4-hydroxybenzoic acid (**6**). After a one-stage Hofmann reaction, beeberine dimethyl ether (**7**) gave a methine base (**8**) which on ozonolysis yielded a mixture of two dimethylamino dialdehydes (**9**) and (**10**). This mixture was methylated, oxidised and boiled with alkali when a mixture of two vinyl dicarboxylic acids (**11**) and (**12**) was obtained accompanied by the liberation of trimethylamine. Compound (**12**) was further oxidised to get the tricarboxylic acid (**13**) whose structure was confirmed by a Ullmann condensation between 4-iodo-5-methoxyphthalic acid (**14**) and isovanillic acid (**15**).

On the basis of the foregoing observations, King formulated d-tubocurarine as (**1**). Thus, by a judicious combination of analytical data, the Hofmann reaction, oxidation and synthetic work, the structure of a fairly complex alkaloid was unambiguously elucidated. The structure was confirmed 25 years later by a total synthesis of (**2**). Condensation of 3-methoxy-4-hydroxy



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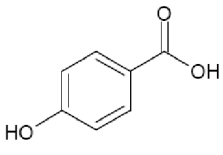

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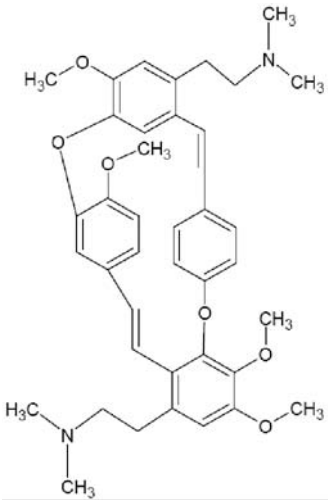

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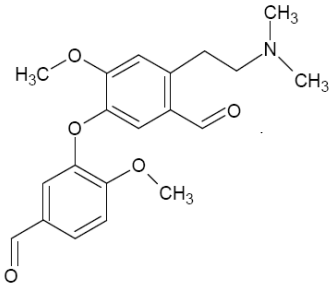

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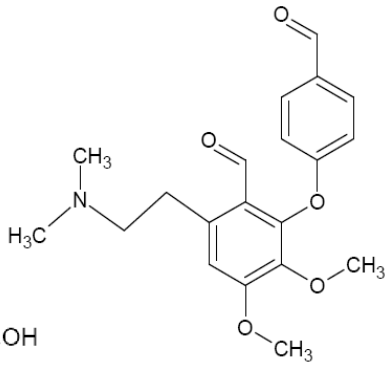

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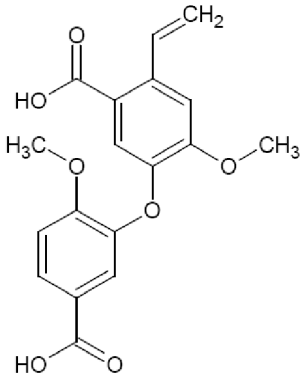

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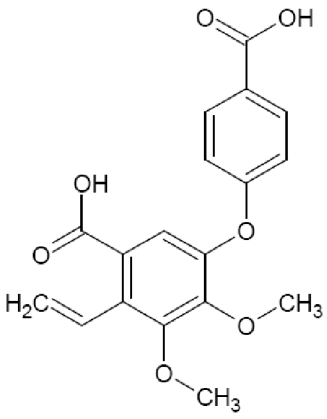

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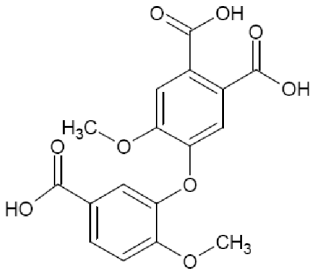

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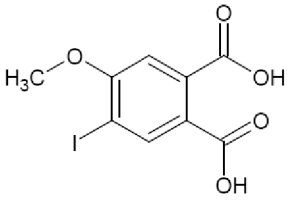

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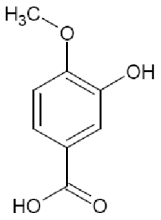

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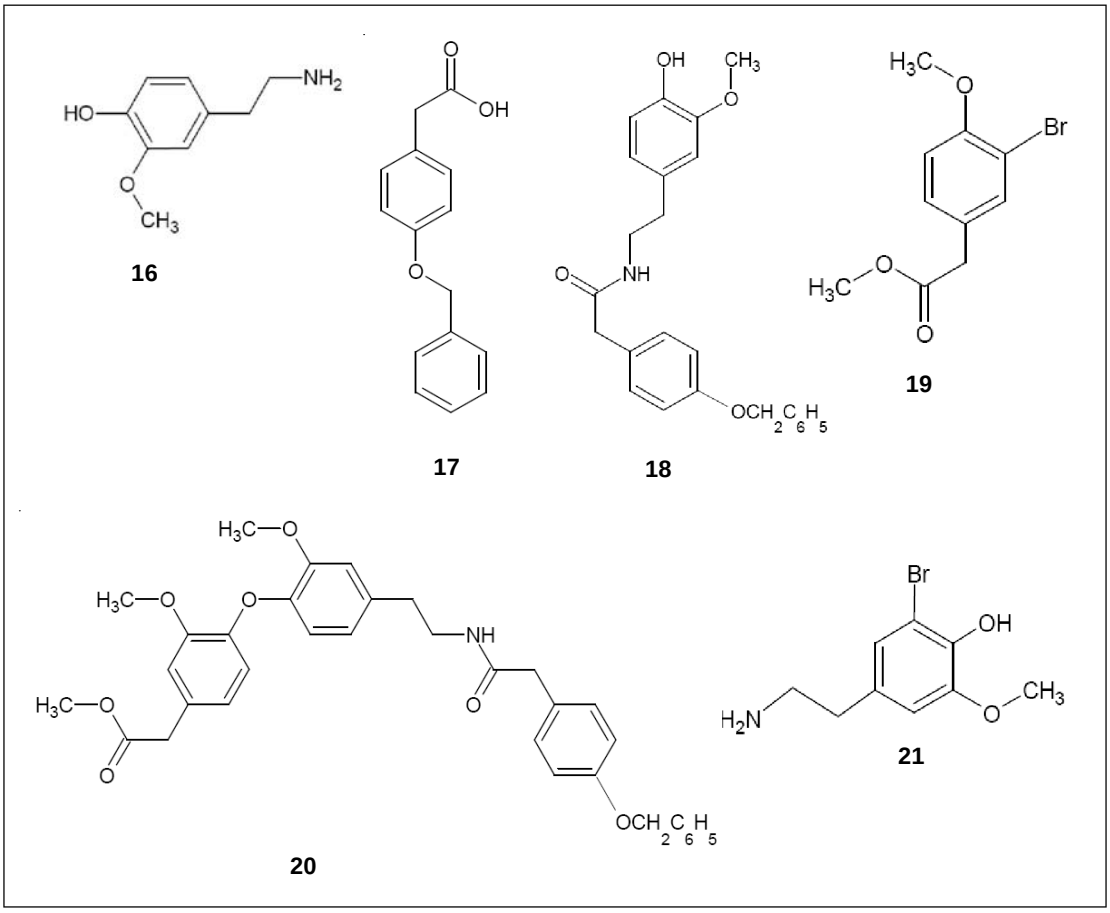
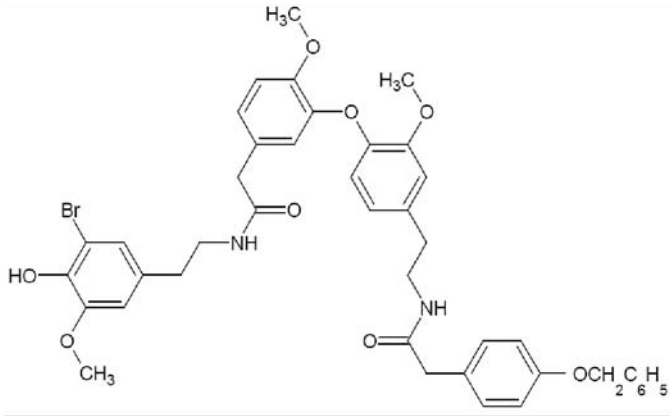


phenyl-ethylamine (**16**) with 4-benzyloxyphenylacetic acid (**17**) gave the amide (**18**) whose potassium salt was heated with methyl 3-bromo-4-methoxyphenylacetate (**19**) in the presence of copper powder (Ullmann reaction). The resulting diphenylether derivative (**20**) was then condensed with 3-methoxy-4-hydroxy-5-bromophenylethylamine (**21**), the product (**22**) methylated and subjected to a Bischler–Napieralski reaction (heating with phosphorus oxychloride). The compound (**23**) thus obtained was debenzylated and then heated with copper powder (intramolecular Ullmann reaction) to get (**24**). The final steps involved reduction of (**24**) with zinc and acetic acid followed by exhaustive methylation with methyl chloride.



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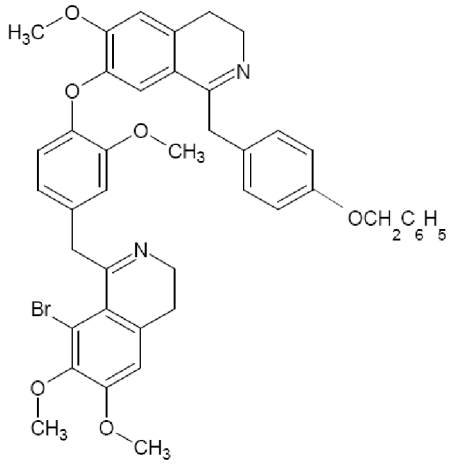

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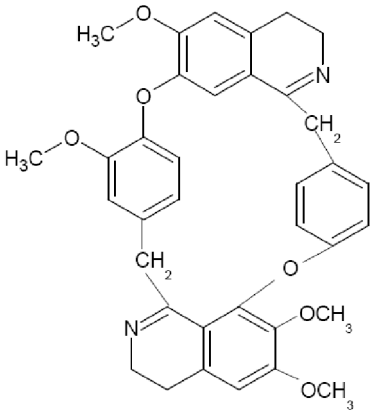

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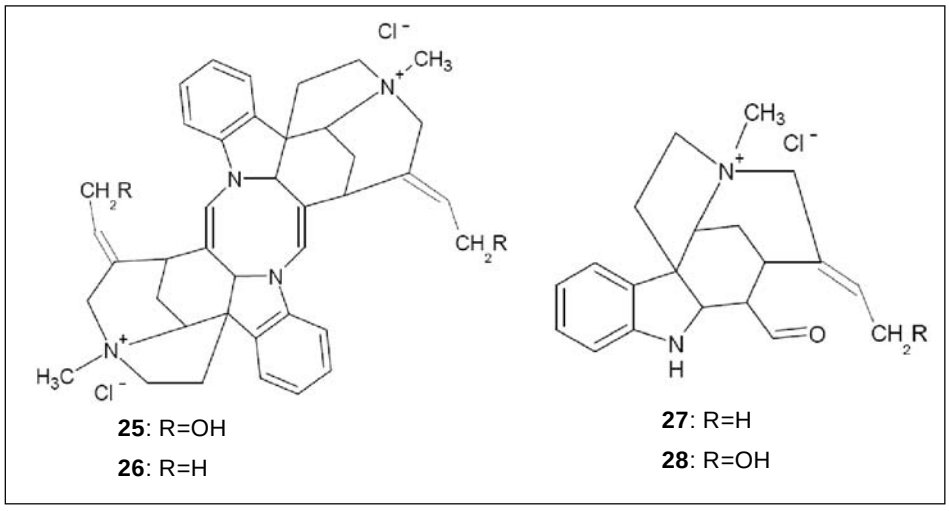
Indole Alkaloids from Calabash Curare

Calabash curare whose major plant constituent is *Strychnos toxifera* is a complex mixture of several indole alkaloids. The first major problem, therefore, was the isolation of the individual chemical components. Initial breakthrough was achieved in the late 1930s and early 1940s by the Munich school headed by H Wieland who used chromatography over alumina to resolve the complex mixture of alkaloids. Later, partition chromatography on cellulose was developed in Munich as well as in the laboratories of P Karrer in Zurich. This enabled the isolation of as many

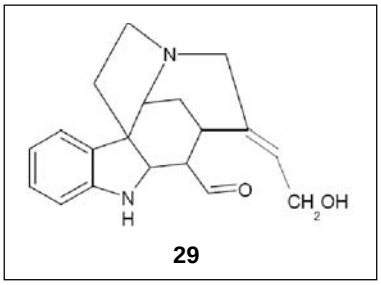
as 40 different alkaloids from a sample of curare of South American origin. Further improvements in the separation methods resulted in the identification of 30 more alkaloids. In this article, however, the chemistry of only the most important calabash curare alkaloid, namely, toxiferine-I, is described as an illustrative example. Besides Karrer and Schmid and their coworkers of the Zurich school and Wieland and his coworkers, significant contributions to the chemistry of these compounds has been made by Battersby and his coworkers.

Toxiferine chloride has the molecular formula $C_{40}H_{46}Cl_2N_4O_2$. Like tubocurarine, it is also a bis-quaternary ammonium compound. It has the structure (**25**). The more abundant alkaloid of calabash curare, dihydrotoxiferine, which is actually deoxytoxiferine (**26**). Initial structural studies were, therefore, carried out with this compound. On treatment with dilute mineral acids, (**26**) gave a C-20 compound, hemidihydrotoxiferine (**27**), which could be converted back to (**26**) by the action of dilute acetic acid. The UV spectrum of hemidihydrotoxiferine (molecular formula $C_{20}H_{25}N_2O$) was typical of indoline derivatives. Its IR spectrum showed the presence of an aldehyde group. Besides toxiferine and dihydrotoxiferine, extracts of *Strychnos toxifera* also contain monomeric indole alkaloids, the chief of which are hemitoxiferine (**28**), a quaternary base which is the N-methyl derivative of the



25: R=OH
26: R=H

27: R=H
28: R=OH



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tertiary base caracurineVII (**29**). Caracurine was later identified as the Wieland–Gumlich aldehyde which was one of the key degradation products of strychnine, the toxic alkaloid isolated from *Strychnos nuxvomica*. Hemitoxiferine is readily obtained from toxiferine on treatment with a dilute mineral acid in the absence of oxygen. Like hemidihydrotoxiferine (**27**), hemitoxiferine (**28**) also undergoes dimerisation in the presence of dilute acetic acid.

Conclusion

The story of curare has elements of social and cultural history of the indigenous people of the Orinoco and Amazon river basins of South America as well as strands from classical organic chemistry and physiology. Eminent scientists including Sir Henry Dale, Paul Karrer and H Wieland have made significant contributions to this fascinating tale. We have left out of this article arrow poisons used in other parts of the world such as Africa and Asia to keep the focus on curare.

Suggested Reading

[1] **N R Krishnaswamy, *Chemistry of Natural Products – a unified approach,* 2nd edition, Universities Press, Hyderabad and CRC Press, Boca Raton, pp.114-118, 2010.**

[2] **H King, *J.Chem.Soc.*, pp.1381, 1935; pp.1157, 1939 and pp.2645, 1948.**

[3] ***Wilson and Gisvold's Textbook of organic medicinal and pharmaceutical chemistry*, 11th edition, Lippincott Williams and Wilkins, Philadelphia, 2004.**

[4] **A R Battersby and H F Hodso, Alkaloids of calabash curare and *Strychnos* species, in *The Indole Alkaloids*, Ed. R H Manske, A G Rodrigo and H Holmes, Academic Press, New York, 1965.**

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