FROM THE "ORDEAL BEAN" (*PHYSOSTIGMA VENENOSUM*) TO THE ORDEAL OF ALZHEIMER'S DISEASE --- SOME OF THE LEGACY OF PERCY LAVON JULIAN* (1899-1975)

Bernhard Witkop

Abstract --- The Calabar bean, *Physostigma venenosum*, contains an alkaloid, physostigmine, which has therapeutic effects in glaucoma, pathological muscle fatigue and presenile dementia (Alzheimer's Disease), either as such or as suitable derivatives, all synthetically accessible by improvements of Percy Julian's first total synthesis in 1935.

On Sunday, April 8, 1990, an Induction Ceremony took place at the National Inventors Hall of Fame in Akron, Ohio: among the ten inductees, such as Hermann Hollerith (1860-1929), inventor of the punched card system, or George Washington Carver (1864-1943), son of a slave and pioneer of agricultural research, was Percy Lavon Julian (1899-1975), grandson of a slave and pioneer in the chemistry of natural products of medicinal importance.

As it says in its Charter:

> "The National Inventors Hall of Fame is dedicated to the individuals who conceived the great technological advances which this nation fosters through its patent system. The purpose of the Hall is to honor these inventors and bring public recognition to them and to their contributions to the nation's welfare."

The invention of the nominee must be covered by a particular patent, in Julian's case U.S. Patent 2,752,339 issued June 26, 1956, entitled "Preparation of Cortisone," a break-through which was published five years earlier in the Journal of the American Chemical Society, {Volume 73, 1982-1985 (1951)}. In a personal letter to the author dated September 28, 1949, Julian refers to the hard labor

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* This retrospection is in commemoration of a lifelong friendship with Percy Julian on the occasion of his hundredth birthday on April 11, 1999, to be honored by the AMERICAN CHEMICAL SOCIETY at their Spring Meeting in Anaheim, California, March 21-25, 1999.

involved in achieving this progress:

> "As you can no doubt imagine, during the past six months I have worked an average of fourteen to sixteen hours daily, including Saturdays, and Sundays, on *partial synthesis* of cortical steroids."

And then he complains that his other work has received scant attention during this period, "a circumstance which I must remedy at the earliest possible moment."

"Pure" and "applied" chemistry are the two worlds that challenge and consume Julian's energy, the former represented in 51 publications from 1931-1969, the latter an impressive record of 117 Patents, issued in the US, France, Great Britain, Germany and Australia. Julian's "other work" that he had to neglect while rushing the synthesis of corticoids steroids, such as sex hormones and anti-inflammatories, goes back to his doctoral thesis on alkaloids done at the University of Vienna under the guidance of Professor Ernst Späth in 1931. His friend Josef Pikl described Julian's interaction with his Viennese fellow students:

> "The two year spent in Austria (1929-1931) had a great influence in developing the personality of Julian. For the first time in his life he was completely at ease, no open or hidden barriers, really an equal among equals. He may even have enjoyed standing a few notches higher than his friends. In the laboratory he was particularly noticed for his neatness, the cleanliness of his work bench, his ready and contagious laugh, completely uninhibited. All fifteen other graduate students in the room were his friends."

This friendship with Joseph Pikl matured into a lifelong association and collaboration, after both returned, first to Howard University in Washington, then to DePauw University in Greencastle, Indiana, where he had entered as a "sub-freshman" from Montgomery, Alabama in 1910. With Pikl he started a vigorous program on the synthesis of one of the most remarkable alkaloids, *viz.* physostigmine, the basic active principle from the calabar bean, *Physostigma venenosum.*

Many alkaloids, such as morphine, nicotine, atropine, quinine, and others are part of the armamentarium of naturally occurring drugs. Physostigmine has a more recent history and has proven its value not only as a therapeutic in controlling glaucoma but also as a tool in elucidating physiological and pharmacological mechanisms (see Table I).

CHRONOLOGY OF PHYSOSTIGMA VENENOSUM

1840	First observation of use of calabar bean in native judicial ordeal procedure	Freeman Daniell (1818-1865)
1855	First toxicology in self-experiments	Sir Robert Christison (1797-1882)
1863	Contraction of the pupil (miosis), antagonized by atropine	Thomas Richard Fraser (1841-1919)
1864	Isolation of crystalline physostigmine	J. Jobst and O. Hesse
1877	First use in glaucoma to lower intraocular pressure	Ludwig Laqueur
1925	Structure of physostigmine (eserine)	Edgar Stedman and George Barger
1921-1926	Inhibition of cholinesterase: discovery of acetylcholine: as neurohumoral tramsmitter	Otto Loewi (1873-1961), Nobel Prize 1936 with Sir Henry Dale (1875-1968)
1935	First total synthesis of physostigmine	Percy Julian
1934	First use of physostigmine for the therapy of Myasthenia gravis	Mary Walker, Edinburgh
1960	Inhibition of choline esterase by transcarbamylation	Irwin B. Wilson
1952	Pest control: carbamate insecticides	H. Gysin (J. R. Geigy)
1986	(+)-Physostigmine protects from nerve gases	Edson X. Albuquerque
1985-1998	Physostigmine analogs for improvement of memory and relief in Alzheimer's disease	Arnold Brossi and the National Institute on Aging, Nigel Graig *et al.*

Table I

What is now the Calabar Province of Nigeria used to be called Old Calabar on the estuaries of the Cross and Niger rivers, inhabited by the Efik tribe. William Freeman Daniell (1818-1865), a medical officer in the British Army who served on the pestilential coast of the West Africa, was the first European to observe the use of the Calabar bean in the strange native judicial procedure, called the *eser ordeal*. The native Efiks believed that the esere or Calabar bean possessed the power to reveal and destroy witchcraft: "A suspected person is given eight of the beans ground and added to water as drink. If he is guilty, his mouth shakes and mucus comes from his nose. His innocence is proved if he lifts his right hand and then regurgitates." This murderous custom was abolished by authority of the British consul in 1878 on the instruciton of Lord Salisbury, then Foreign Secretary in a treaty with the king and chieftains of Duke Town consisting of 15 articles:

> Article three: Any person administering the esere bean, whether the person taking it dies or not, shall be considered guilty of murder, and shall suffer death.
>
> Article four: Every person taking the esere bean willfully, either for the purpose of committing suicide, or for the purpose of attempting to prove their innocence of any crime of which they may have been accused, shall be considered guilty of ATTEMPTED MURDER AND SHALL BE BANISHED FROM THE COUNTRY.

Even today the laws of Nigeria forbid the possession or use of the Calabar bean.

The scientific exploration of the Calabar bean started in Edinburgh in 1846 where the keeper of the Royal Botanical Gardens, John Hutton Balfour (1808-1884) had contact with the Scottish missionaries of Old Calabar, especially with the reverend Hope Masterton Waddell (1804-1895) who brought Calabar beans to Edinburgh where they grew in Balfour's garden but never flowered. An Edinburgh toxicologist, Sir Robert Christison (1797-1882) was the first to describe the effects of the bean in an experiment on himself which he published in the Monthly Journal of Medicine (London) in 1855 under the title "On the Properties of the Ordeal Bean of Old Calabar." His doses in two experiments were first six then forty-eight grains of the powder of ground Calabar beans.

Christison's assistant and later successor (in 1877), Thomas Richard Fraser (1841-1920) made the important discoveries that the local application of extracts from the Calabar bean to the eye ball led to miosis or contraction of the pupil, an effect which could be counteracted and prevented by atropine. Fraser thus became the discoverer of "The antagonism between the actions of active substances" which he published in the British Medical Journal in 1872. As early as 1863 Fraser had alerted his friend, the ophthalmic surgeon, Douglas Argyll Robertson (1837-1909), to the use of the Calabar bean in ophthalmic surgery. Robertson's interpretation of the contraction of the *sphincter pupillae* was ahead of its time, because he regarded this effect as a stimulation of the ciliary nerves. In 1877 Ludwig Laqueur was the first ophthalmologist to recommend physostigmine or eserine for the relief of glaucoma, because the

alkaloid lowered intraocular pressure temporarily or permanently.

The Discovery of Neurohumoral Transmission: Physostigmine and Choline Esterase.

Otto Loewi (1873-1961) in 1911 observed that physostigmine increased the sensitivity of peripheral organs to electric stimulation. At that time the question, "How are nerves stimulated ? Electrically or chemically ?" could not be decided. The answer, literally, came in a dream that Loewi had in 1921:

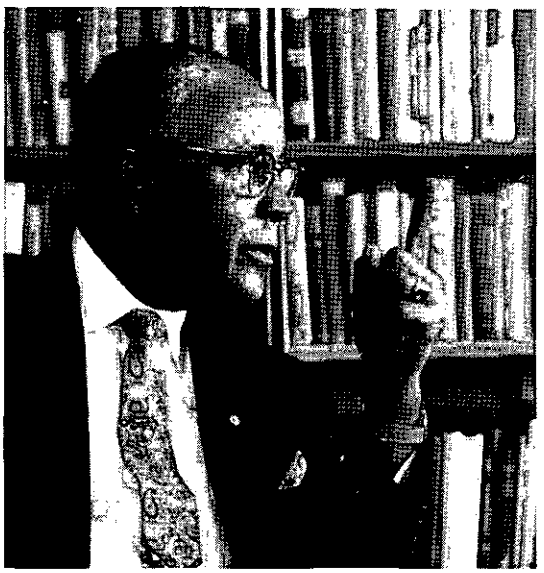
> The night before Easter Sunday of that year (1921) I awoke, turned on the light and jotted down a few notes on a tiny slip of paper. Then I fell asleep again. It occurred to me at six o'clock in the morning that during the night I had written down something most important but I was unable to decipher the scrawl. The next night, at three o'clock, the idea returned. It was the design of an experiment to determine whether or not the hypothesis of chemical transmission that I had uttered seventeen years ago was correct. I got up immediately, went to the laboratory, and performed a simple experiment on a frog heart according to the nocturnal design. I have to describe this experiment briefly since its results became the foundation of the theory of chemical transmission of the nervous impulse.
>
> The hearts of two frogs were isolated, the first with its nerves, the second without. Both hearts were attached to Straub cannulas filled with a little Ringer solution. The vagus nerve of the first heart was stimulated for a few minutes. Then the Ringer solution that had been in the first heart during the stimulation of the vagus was transferred to the second heart. It slowed and its beats diminished just as if its vagus had been stimulated. Similarly, when the accelerator nerve was stimulated and the Ringer from this period transferred, the second heart speeded up and its beats increased. These results unequivocally proved that the nerves do not influence the heart directly but liberate from their terminals specific chemical substances which, in their turn, cause the well-known modifications of the function of the heart characteristics of the stimulation of its nerves.

This experiment led to "one of the most remarkable communications in the history of science" (Bo Holmstedt), entitled "Über humorale Übertragbarkeit der Herznervenwirkung", published in Pflügers Archiv der gesamten Physiologie in 1921. Based on earlier observations of Reid Hunt (1870-1948) that the effects of acetylcholine were intensified by eserine or physostigmine, Loewi cautiously concluded that "acetylcholine, of all the substances examined, of being the natural neurohumoral transmitter is the one most suggestive." Sir Henry Dale (1875-1968) added the missing link to this chain of thought: "It seems not improbable that an esterase contributes to the removal of the active ester from the circulation and

the restoration of the original condition of sensitivity." This was the first mention of an esterase (enzyme) in connection with the brevity of action of acetylcholine. In the eleventh paper in his famous series, "The mechanism of the effect of physostigmine on acetylcholine", Loewi gave this view its experimental basis.

Structure and Synthesis of Physostigmine.

In order to understand this unique inhibitory activity of physostigmine on an important enzyme, the structure of this alkaloid had to be known. After its isolation in pure and crystalline form in 1864, famous chemists, such as the brothers Polonovski (1893-1923), Stedman and Barger (1925), and Robert Robinson (1932-1935) worked on its elucidation. The final proof of structure came when Percy Julian and Josef Pikl published a paper in 1935 entitled "The Complete Synthesis of Physostigmine" in which they state:



Physostigmine, the principal alkaloid of the Calabar bean, and long used as a drug, has, since its isolation by Jobst and Hesse 70 years ago, been the subject of numerous investigations. The determination of its constitution was rendered particularly difficult since its peculiar chemical structure found no analog in other plant products of known composition....Shortly after promising experiments in the direction of (its synthesis) were under way (in our laboratories) the work had to be interrupted and could only be resumed recently. In the meantime, the first of a series of ten papers dealing with the synthesis of Physostigmine, by Robinson and his collaborators, appeared and seemingly proved convincingly that the (course) suggested in our formulas could not be realized in practice. Our experiments, nevertheless, were continued and led to the successful synthesis of d,l-Eserethole.....

To our surprise, our (d, l-Eserethole) exhibited entirely different properties than those of a compound synthesized by Robinson and his co-workers and called "d,l-Eserethole". Likewise were all derivatives different. Inasmuch as our (optically) inactive material, subjected to characteristic reactions of Eserethole of natural origin, yielded perfectly analogous results, we expressed the belief that our product was the real d,l-Eserethole. This is now proved conclusively by synthesis of l-Eserethole, identical with the product of natural origin.

Friends of Percy Julian, such as his teacher at Harvard, Elmer Peter Kohler (1865-1938), or Max Tishler (1906-1989), the president-to-be of Merck, Rahway, New Jersey, all held their breath, because here were *two brash neophytes challenging the work of Sir Robert Robinson* (1886-1975, Nobel Prize 1947) an eminent British chemist. But Julian was right and Robinson wrong. Telegrams of congratulations came from all parts of America, Europe and Asia.

Julian's admires in Asia, even sixty years later, still praise his achievements and when Julian finally was issued his commemorative stamp on January 29, 1993, in the series "Black Heritage", this was duly noticed in Japan by the late Professor Yoshio Ban (1921-1994):

▲
1993年1月に発売された
"Percy Lavon Julian" 記念切手

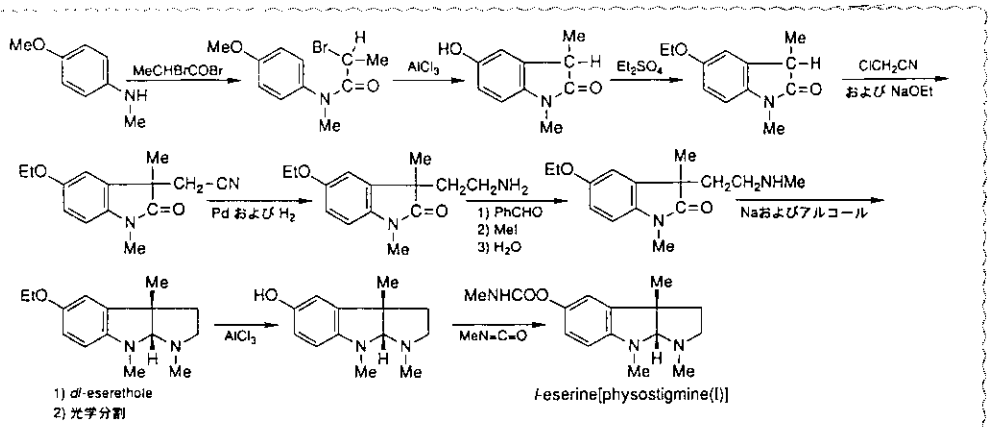
Percy Julian物語

ある有機化学者の足跡と交友

伴　義雄（ばん　よしお）
〔北海道大学名誉教授/薬品製造学〕

これは第二次世界大戦前にアメリカの生んだ傑出した黒人有機化学者 Percy Lavon Julian 博士(1899～1975)の想像を絶する悪戦苦闘の生涯と，その彼を長年にわたって支え続けた Witkop 博士の心暖まる友情の物語である[1~3].

Although there have been more than half a dozen subsequent syntheses of physostigmine, Julian's classical approach, with some recent modifications, is still the best route to the synthetic alkaloid and, therefore, is duly remembered in the latest eulogy from Japan:



● 図1 Julian と Pikl による *l*-eserine の全合成 ●

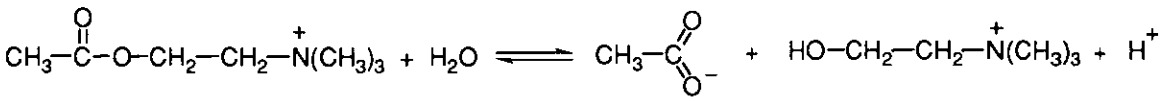
More recently there has been a growing need for new derivatives of physostigmine in clinical trials and applications, and, therefore, has focused interest on synthesis on a larger scale. Like most natural products physostigmine has asymmetry or chirality and exists in the plant as the (laevorotatory) (-)-isomer. This natural form (optical (-)-isomer) is highly potent in inhibiting the endogenous cholinesterase enzymes, whereas the unnatural (+)-antipode is inactive, but has other useful properties. Total synthesis leads to a racemic mixture of both optical isomers which have to be separated (resolved) at the right stage by the right method. This problem was recently tackled and solved in a collaboration of the National Institutes of Health, Georgetown University and the Shanghai Institute of Organic Chemistry under the guidance of Arnold Brossi [cf." Heterocycles", Volume 36, 1279 (1993)]. The stage of resolution takes place early in the sequence of reactions, and the racemic precursor, oxindole-acetic acid, provides the optically pure intermediate.

In remembering Julian's synthesis (1935) his friend and collaborator Josef Pikl, since deceased, in a personal letter to the author (1977) gives a vivid description of the research climate and the hard work that made the original total synthesis such a success:

> Throughout the six years of our collaboration, we made a good team. Percy generated ideas faster than half a dozen people could critically review and test them. He also did most of the writing, did practically all of the analytical work, such as carbon-hydrogen analyses, and determination of active hydrogen with his Grignard machine, and helped with much of the dish-washing chores using a two foot diameter porcelain dish with hot sulfuric acid and nitric acid, unaware of the dangers of this method, outside of acid burns. When we were celebrating some progress or the receipt of a nice letter, we drove out about six miles to the crossing of the Transcontinental Route 40 where there was a small snack restaurant. Usually, however, we stayed up to 11-12 o'clock in the laboratory so that we heard some complaints of burning too much midnight oil !

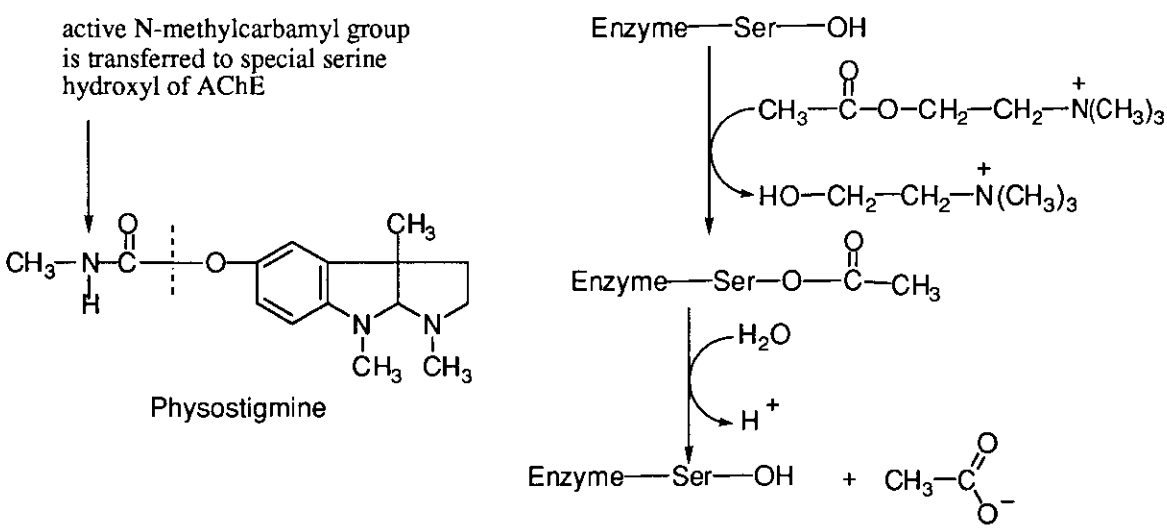
Carbamylation of the Acetylcholinesterase by Physostigmine.

The action of the neurotransmitter acetylcholine is terminated by hydrolysis to acetate and choline by the enzyme acetylcholinesterase (AChE) :



Acetylcholine Acetate Choline

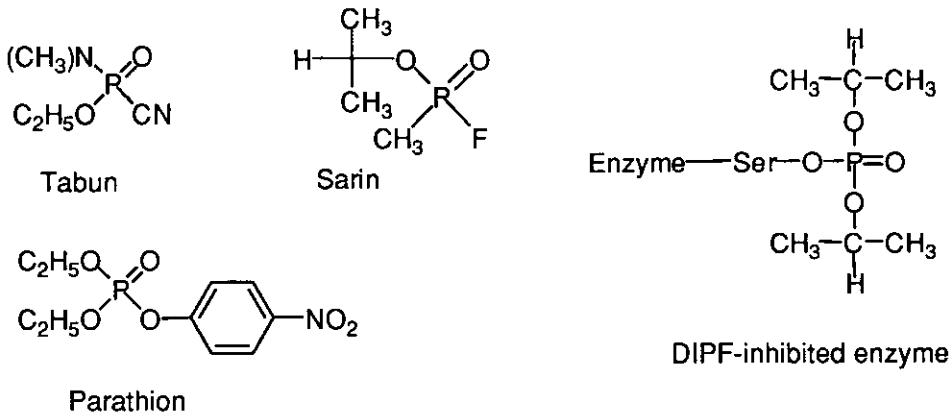
AChE was discovered by David Nachmansohn (1899-1983) at Columbia College of Physicians and Surgeons in 1938. This enzyme and its close relative, butyrylcholinesterase (BChE), are found throughout the body and appear to have more than one function. The critical role of AChE in neurotransmission is indicated by its localization in the synaptic cleft of cholinergic neurons where AChE is bound by a network of collagen and glucosaminoglycans to the post-synaptic terminal. The catalytic activity of this enzyme is remarkable and is expressed by its very high turnover number of 25,000, which means that AChE cleaves a molecule of acetylcholine in 40 microseconds, so that synapses can transmit 1000 impulses per second, provided the membrane recovers its normal polarization (resting potential) within fractions of a milli-second. The catalytic mechanism of AChE resembles that of other so-called "active serine esterases", such as chymotrypsin: Acetylcholine reacts with a specific serine residue at the active site of AChE to form a covalent acetyl-enzyme intermediate, and choline is released. This acetyl-enzyme intermediate then rapidly reacts with water to form acetate and regenerate the free enzyme.



Catalytic mechanism of acetylcholinesterase

The Unnatural Antipode, (+)-Physostigmine Protects against Nerve Gases.

The slow rate of hydrolysis of carbamoly-AChE intermediates is far surpassed by the almost irreversible combination of the so-called "nerve gases", such as Tabun, Sarin, Parathion or di-isopropylfluoro-phosphate (DIPF).



Organic phosphate inhibitors of acetylcholinesterase

During the years of the cold war with all the anxiety about chemical warfare the search for an effective antidote led to a surprising discovery: The unnatural (+)-physostigmine had been known for some time (1970, 1993) but it acquired a new importance when, in conjunction with atropine, the well-known basic principle of the Belladonna plant, it offered prophylactic protection, less through interaction with AChE, but by combining with a special site on the (nicotinic) acetylcholine receptor site. Thus (+)-physostigmine is no longer a useless byproduct of the total synthesis of the natural base, but useful for new and exciting studies of the AChE-receptor (Edson X. Albuquerque, University of Maryland Medical Center).

Physostigmine Therapy in Myasthenia Gravis.

There is a disorder of neuromuscular function, clinically observable through muscular debility, fatigue and exhaustion of part or all of the muscular system, thought to be due to the presence of antibodies to (nicotinic) acetylcholine-receptors at the neuromuscular junctions, in other words an auto-immune disorder. This disease afflicts at least one in 10,000 persons and can occur at virtually any age, but is observed more often in women in their twenties and thirties, and in men in their fifties and sixties. It is a long and

disabling disease whose fundamental defect is a decrease in the number of available acetylcholine receptors at postsynaptic muscle membranes which are altered by being flattened and with much fewer folds than normal. Although acetylcholine release proceeds in a normal way from the pre-synaptic terminal, it produces only a small end-plate potential which often fails to cause depolarization and does not trigger muscle fibers into contraction. Whereas the clinical symptoms of this syndrome have been known for more than a hundred years, the first publication linking this disorder with physostigmine did not appear until 1934 in Lancet under the title "Treatment of *Myasthenia Gravis* with Physostigmine." The author, Mary Walker, had received her medical degree at the University of Edinburgh, the place that is so intimately connected with the history of physostigmine. The first positive clinical results are best described in her own words:

> The abnormal fatigability in *Myasthenia Gravis* has been thought to be due to curare-like poisoning of the motor nerve-endings or of the "myoneural junctions" in the affected muscles. It occurred to me recently that it would be worth-while to try the effect of physostigmine, a partial antagonist to curare, on a case of *Myasthenia Gravis* at present in St. Alfege's Hospital, in the hope that it would counteract the effect of the unknown substance which might be exerting a curare-like effect on the myoneural junctions. I found that hypodermic injections of physostigmine salicylate did have a striking though temporary effect.

Subsequent improvements with other patients were refereed to as "the miracle at St. Alfeges", the hospital in Greenwich, England, where Dr. Walker was working. In 1935 she was awarded the gold medal of medicine from the University of Edinburgh; in 1955 she became an honorary member of the Myasthenia gravis Foundation, an organizer of frequent international symposia.

Drugs that inhibit AChE and increase the physiological half-life time of released acetylcholine for longer interaction with the limited number of acetylcholine receptors improve muscle strength and remain, to the present day, the major therapeutic approach. Pyridostigmine and Neostigmine, two analogs of physostigmine that do not enter the brain, are two of the most prescribed inhibitors of AChE and provide relief for clinical symptoms of *Myasthenia Gravis*.

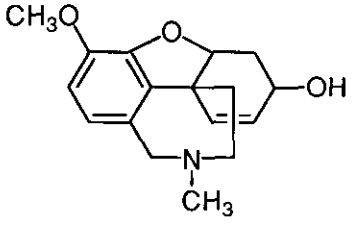
Physostigmine Analogs and Alzheimer's Disease

The loss of memory during senescence and cognitive deficits, what is referred to as senility, is a phenomenon that the German neurologist Alois Alzheimer (1864-1915) observed in people as young as 40 years and called *presenile dementia*. In its severe form this syndrome is characterized by cortical atrophy and major histological degenerative changes in the brain. The disease is the fourth leading cause of death in Western Societies, afflicting some 4 million American and an additional seven million in other

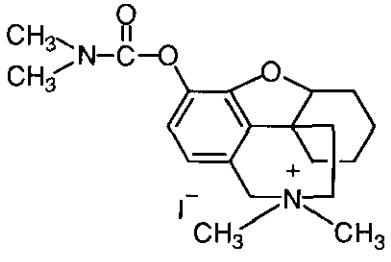
developed countries. Mental disorders, such as schizophrenia, mostly have a biochemical or neurophysiological etiology and their study has always led to new insights into normal and abnormal metabolism of neurohumoral transmitters. Thus, attention has been focused on the role of the cholinergic system in the behavior of normal humans compared with Alzheimer patients: it is now recognized that deficiences in the cholinergic system may account for some of the cognitive deficits, i.e., loss of memory, aphasia, etc., found in Alzheimer patients. Early in the course of the disorder, the forebrain cholinergic system of neurons, which activates crucial brain areas by the release of acetylcholine, becomes progressively impaired. Yet, these neurons are critical for learning and memory as evidenced by their transient inhibition, either by drugs in man, or their experimental destruction in animals, both leading to profound amnesia. Degeneration of this neuronal system is one of the first observable changes that occurs during Alzheimer's disease, and the degree of degeneration is directly correlated with the severity of memory impairment.

Neuorchemcial studies indicate that, although there is a dramatic loss of presynaptic elements of the cholinergic system in brain, especially in the hippocampus and cortex, brain areas associated with memory and cognition. The postsynaptic elements remain relatively intact except late in the disease when virtually all neurotransmitters are at lower level and there is a loss of brain matter.

Understandably the search for therapeutic agents immediately concentrated on suitable inhibitors of AChE, such as physostigmine, the alkaloid galanthamine or its quaternary dimethylcarbamoyl analog, or 5-amino-tetrahydroacridine (Tacrine or Cognex). Such an inhibitor should satisfy the following requirements:



Galanthamine



Deoxydemethyllycoramine
N,N-dimethylcarbamate
methiodide

1. produce a long-term and selective acetylcholinesterase inhibition in brain with a steady-state phase of increased cortical acetylcholine.

2. not inhibit acetylcholine synthesis or its release in nerve endings.

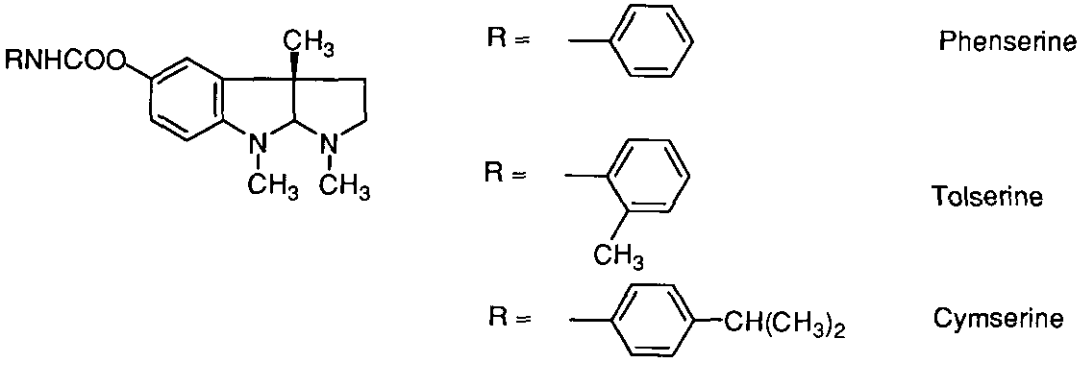
3. produce no or only mild side effects at therapeutic doses.

Compund	Anticholine esterase act.	Selectivity: AChE/BChE	Pharmaco-dynamics: Duration of Action (min)	Pharmaco-kinetics: Physiological Half-life Time	Brain Level	Metabolic Clearance	Therpeutic Window
Physostigmine	high	none	30	10-30 min.	none	slow	narrow
Tacrine (listed for control)	moderate	none	90-120	30 min.	none	slow	narrow
Heptyl-physostigmine	high	none	400	5 hours	N. A.	medium	moderate
Phenserine	high	high	400	10 min.	high	fast	broad
Ideal drug postulated by theory	high	high	long	short	high	fast	broad

Table II. Julian's historical synthesis of physostigmine, with some modifications, is still used as an approach to suitable derivatives as candidates for the relief of degenerative brain disorders, such as Alzheimer's disease for which the observed and ideal requirements are listed.

Both physostigmine and tacrine have been administered to Alzheimer patients to assess their therapeutic value as inhibitors of AChE. Both drugs improved cognition in the majority of patients; however, they have side effects, such as short duration of action, high toxicity and unselective action on both forms of the enzyme, AChE and BChE. Cognitive improvement is commensurate with the level of inhibition of AChE. Unfortunately, for both drugs, peripheral side effects became dose-limiting early in the treatment, a limitation which has hampered the true evaluation of ACE-inhibitors for Alzheimer's disease. Other candidates for experimental therapy, such as the highly lipophilic heptastigmine, an N-heptylcarbamyl-physostigmine, causes agranulocytosis with the consequence that its clinical trials have been discontinued.

Here we return to Julian's first total synthesis and its recent improvement (*vide infra*) and concentrate on phenylcarbamates of physostigmine:



Phenserine, as a result of its physico-chemical properties, has optimal characteristics for central nervous system (CNS) activity. The substitution of the methylcarbamyl of physostigmine by the phenylcarbamyl group increased its lipophilicity seven-fold and hence its ability to cross the blood-brain barrier.

The Action of Anticholinestrases, and Specifically of Phenserine, in Alzheimer's Disease.

In Alzheimer's disease, cholinergic inputs to higher brain centers are reduced in number, leading to a reduced release of neurotransmitter, acetylcholine, and, as a consequence, a reduced stimulation of postsynaptic elements. By inhibiting acetylcholinesterase (localized on the postsynaptic cell surface) and blocking neurotransmitter destruction, anti-cholinesterases prolong the life of acetylcholine, restoring

levels in the synapse. Phenserine is delivered preferentially to the brain, where it selectively inhibits acetylcholinesterase, and therefore is a promising drug candidate for the treatment of Alzheimer's disease.

Whereas phyosostigmine and tacrine enter the brain from the systemic circulation and maintain a brain/plasma ratio of 1:1, phenserine and its analogs achieve and maintain ten-fold higher brain levels compared with plasma; they act selectively on ACE rather than BChE, have minimal peripheral side-effects and a duration of inhibition of AChE of more than 8 hours compared with 30 minutes for physostigmine. Interestingly, once the enzyme has been inhibited, a continued presence of the drug for pharmacological activity is no longer required and phenserine and its analogs disappear rapidly from the body. The long duration of action of these compounds, their pharmaco-dynamics, results in a regimen of low dosing frequency and their rapid clearance (pharmaco-kinetics) from the blood stream with a low exposure of the body to the drug, two highly desirable attributes for a drug candidate for the elderly generation.

Not only do brain levels of acetylcholine rise in the rat brain after administration of phenserine, but such an unusually wide "therapeutic window" has not been seen in other drugs when it comes to behavioral tests as a cognition enhancer in rats. Specifically, phenserine improves the ability of young and aged animals to learn a complex maze pattern in which correct choices are required to negotiate through it. The improved memory in aged rats not only suggests that the drug could be useful in Alzheimer's disease but also as a potential remedy in age-associated memory disorders.

Epilog.

Physostigmine, the first alkaloid proven to act through inhibition of an enzyme, and its analogs are used as miotics in glaucoma, as therapeutic agents in *myasthenia gravis*, intestinal paralysis, antagonists of curare in anaesthesiology, as antidotes in atropine poisoning, and now in the form of phenserine or tolserine as promising candidates for the treatment of Alzheimer patients. If Percy Julian were alive and could observe the growing importance of physostigmine and the blessing that his seminal first total synthesis left to coming generations, he would probably exclaim with Macbeth: "If you can look into the seeds of time and say which grain will grow and which will not."

Physostigma venenosum - the Bridge to Stigmasterol and the Sex Hormones.

Julian's first U.S. Patent 2,218,971 was granted on October 22, 1940 and entitled "Recovery of Sterols." Max Tishler, President of Merck to be, in 1965 described the chain of events:

> And then came one of those bits of "accidental chemistry." In attempts to isolate

> Geneserin, a companion alkaloid of Physostigmine, from the Calabar bean (*Physostigma venenosum*), Julian had first extracted the oil from this rather lovely bean. The oil had been washed with dilute acid and then with water, and was set aside wet. On examining it some weeks later, glistening small crystals had separated. They were carefully separated from the oil and found to be a hydrate, which upon losing its water, was again soluble in the oil. After careful recrystallization of the minute quantity of dehydrated material, microanalysis showed the formula $C_{29}H_{48}O$. A literature search showed that it was the sterol, stigmasterol, named after the plant *physostigma venenosum*, from which Windaus and Hauth had separated it 29 years before.

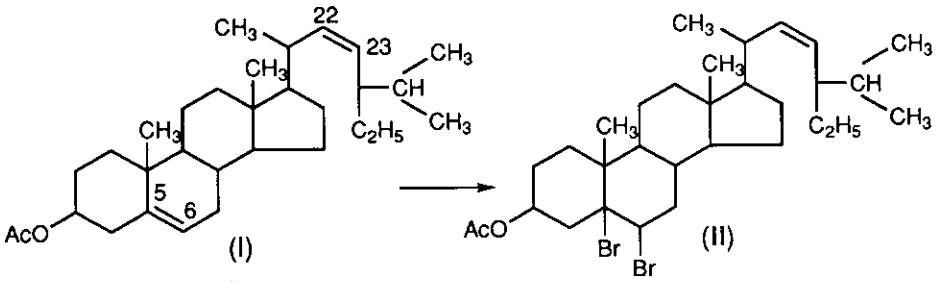
Butenandt and his collaborators Mamoli, Fernholz, Westphal, had converted stigmasterol into the sex hormones pregnenolone and progesterone, hormones of great commercial value. The most convenient source for the isolation of stigmasterol was soy bean oil. When Julian asked the Glidden Company in Chicago for a 5-gallon sample of soy bean oil, Mr. W. J. O'Brien, the Vice President offered him the position of Glidden's Assistant Director of Research of the Soya Product Division, an offer that Julian gladly accepted in 1936.

In the following 4 years this Soya Products Division became Glidden's most profitable single entity as evidenced by Julian's many patents, such as "Lecithin Granules" from Soya Phosphatides, or liquid shortening and Durkee's edible emulsifers and new animal feeds.

And then came the sequence to the happy accident that Tishler referred to: One day workers called Julian, as chief trouble shooter, to advise what should be done with a 100,000 gallon tank of purified soy bean oil into which water had leaked and which contained a mass of white solid. Julian remembered his experience at DePauw, had the whole tank centrifuged and obtained about 15% of mixed soya sterols, amounting to about 100 pounds of sterols daily with a value of about $10,000 in 1940. Julian lost no time and, on an unprecedented industrial scale, ozonized 100 pounds daily of sterol dibromides to end up with pure progesterone, pure female sex hormone. In 1948 Philip Hench (1896-1965), Edward Kendall (1886-1972) who in 1950 shared the Nobel Prize with Tadeus Reichstein (1897-1996), made the eopochal discovery that cortisone, then called Kendall's Compound E, reversed the symptoms of rheumatoid arthritis. Julian lost no time and published the synthesis of Reichstein Substance S (*vide supra*), which differs from cortisone in lacking only an oxygen (in position 11). A personal letter from Julian to the author dated July 22, 1957, describes this time of hard work and great stress:

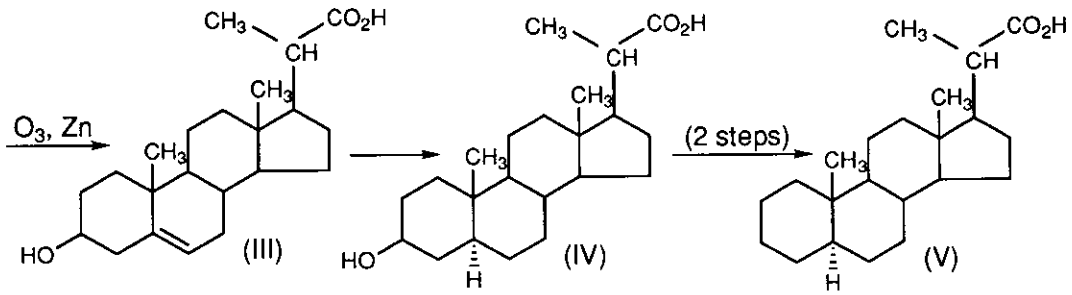
> "In the meantime, during the critical building years of Julian Laboratories, the last three years, I have had to become a businessman and have had very little time to devote to any researches other than our steroid researches with various clients, particularly with Smith, Kline and French Laboratories. Now that Julian Laboratories have become a success

Stigmasterol; Structure of the Side Chain



(I) (II)

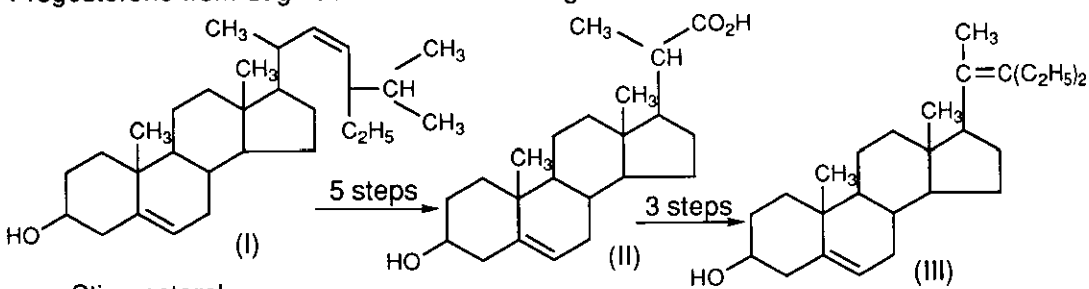
Stigmasteryl acetate



(III) (IV) (V)

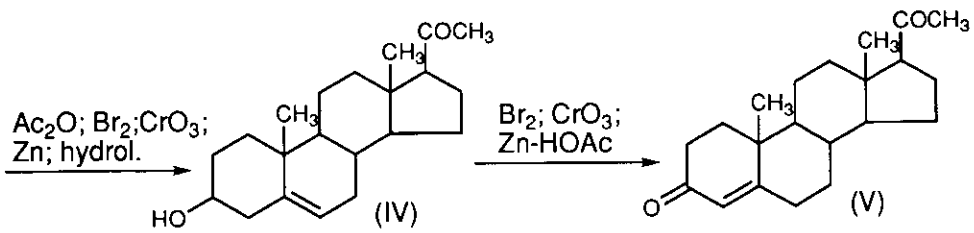
Bisnorallocholanic acid

Progesterone from Stigmasterol and from Pregnanediol



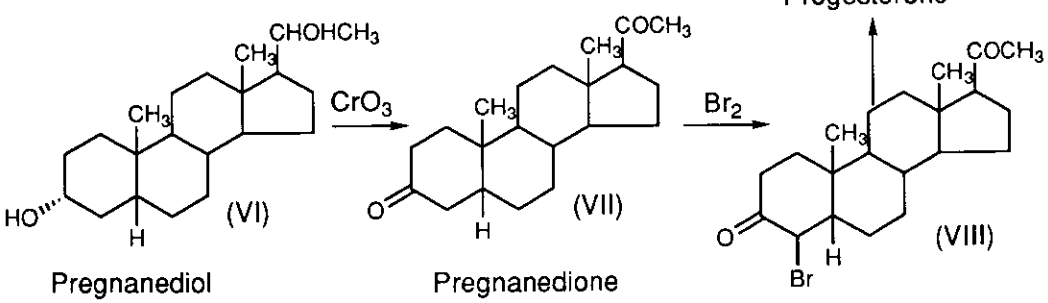
(I) (II) (III)

Stigmasterol Hydroxybisnorcholenic acid



(IV) (V)

Progesterone



(VI) (VII) (VIII)

Pregnanediol Pregnanedione

(for your confidential information our accountants have just given me our sales for the first 10 months of our fiscal year ending August 31st, and they show close to 1.5 million dollars), I am again able to turn back to some of the things which have interested me, of course, most of my life. I still am far from being out of the woods. I need more men badly, among them a good plant manager and good production superintendent, several research men, two more Ph.D.'s at least and three or four junior assistants, a new personal assistant, etc., etc. Incidentally, in this connection, I would appreciate it if you know of any young men who might like to join our organization. As you can probably imagine, we have a very ambitious research program going on in the steroid field, some of which will, no doubt, be published shortly, now that the necessary patents and the necessary protections for our clients have all been cared for. You may also be interested to know that we import from our plantations and our factory in Central America, Dioscorea root, process it into Diosgenin, and into 16-Dehydropregnenolone here at Julian Laboratories, and thus have become very competitive, and the field a bit overcrowded, we have enjoyed a very good business, and are looking forward to double our sales for the coming year, now that our raw material supply is adequate. I hope that in the not-too-distant future, you can visit our laboratories here and see our research and production set-up."

Concluding Eulogy.

While he was alive, and even posthumously, Julian was rewarded by 19 honorary degrees, one of them at Oberlin college in June 1964, with the following eulogy:

In these days in which specialization sometimes seems to dominate, I have the privilege of presenting a man who illustrates the general usefulness of an educated mind. In sequence as chemistry teacher, teacher and researcher, researcher and administrator, and entrepreneur and researcher, Percy Julian joined several careers through his continuing interest in natural materials from plants.

He demonstrated his chemical competence and creative imagination in applied chemistry by securing a number of patents for the making of desired substances from the plant products, but he also kept on publishing in pure chemistry an impressive series of papers on indoles, sterols and steroids, and conjugated systems. Finally, he founded two firms through which he could apply his scientific knowledge, inventive skill, and judgment to recover large quantities of intermediate substances from soya beans and other plants and to make from them hormones and other drugs at low cost. We honor him for his humane objectives.

ACKNOWLEDGMENTS

The author is indebted to Percy Julian and his family for a friendship that goes back to Julian's synthesis of physostigmine, his studies on oxytryptophan, indoles and yohimbine, that is a time span of almost sixty years which produced an ample correspondence, now in the archives of the Chemical Heritage Foundation in Philadelphia. Liberal use is made of "The Ordeal Bean of Old Calabar: The Pageant of *Physostigma venenosum* in Medicine", the comprehensive review by Bo Holmstedt, cf. Plants in the Development of Modern Medicine, Tony Swain editor, Harvard University Press, 1972, pp. 303-360. Arnold Brossi kindly made available his most recent Review "Phenserine and Ring-C Hetero-Analogs: Drug Candidates for the Treatment of Alzheimer's Disease, Medicinal Research Reviews, **15**, No.1, 3-31(1995), which Dr. Nigel Greig, National Institute of Aging kindly adapted for this article. The monograph "Cholinergic Baisis for Alzheimer Therapy", Robert Becker and Ezio Giacobini, Birkhäuser, 1991, was a valuable source of information. Dr. Robert S. Miner, one of Julian's closest friends, shared his collection of documents and photographs most generously.

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