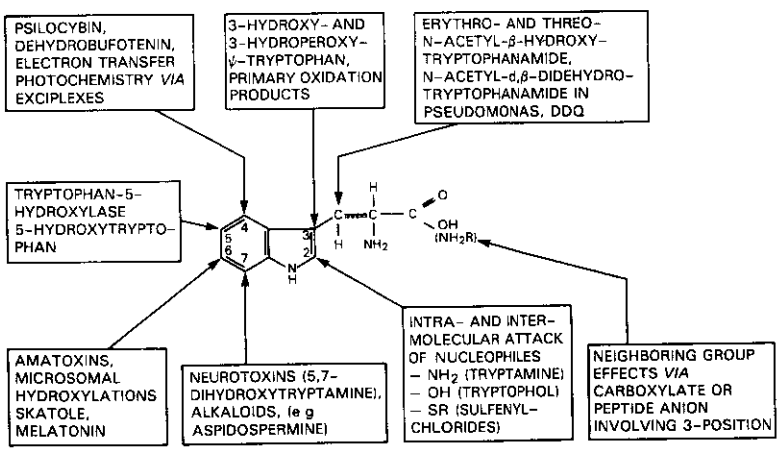
FORTY YEARS OF TRYPTO-FUN*

Bernhard Witkop

National Institutes of Health, Bethesda, Maryland 20205, USA

Abstract - Almost every atom in free or bound tryptophan is capable of reacting selectively under appropriate conditions, both in vitro as well as in vivo. An organic chemist's approach to this challenging problem in differential and selective reactivity, covering a span of more than forty years, is presented with due emphasis on the historical contributions from Japan before and during the NIH Visiting Program, started more than a quarter century ago.



More than any other amino acid tryptophan (the word means "what appears by digestion") has probably the largest number of entries in Index Medicus, many of them from Japanese laboratories, such as the one of Y. Kotake or that of Osamu Hayaishi, Tohru Hino, Osamu Yonemitsu, Yuichi Kanaoka, Teruo Matsuura, Fumio Sakiyama and others with all of whom I had the pleasure to discuss the metabolic fate of tryptophan over many years.

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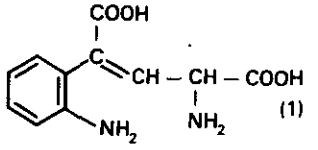
*Dedicated to the memory of Munio Kotake (1894-1976) who, unknowingly , started the Visting Program at NIH with his letter of April 27, 1950, reproduced here for the first time.

TRYPTOPHAN, AN AROMATIC ENAMINE

Many of the puzzling reactions of tryptophan become interpretable when the 2,3-double bound is accorded some independence from the intact aromatic system and is viewed as a mobile enamine system with all the diversity and reactivity known for enamines.[1] Thus, in retrospect, it is obvious that Y. Kotake's classical structure for kynurenine (1)[2], a major metabolite of tryptophan (2), had to be revised to the phenone (3), an event that occurred in 1943[3] when Butenandt investigated the sequence that leads from tryptophan to ommochromes, the ocular pigments of insects.[4] His convential 5-step synthesis was simplified by taking advantage of the reactivity of the 2,3-double bond toward oxidants, such as ozone, which converts (protected) tryptophan to formyl-kynurenine in a one-step operation[5] that has been improved over the years.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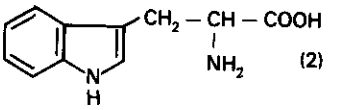
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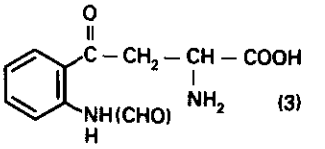
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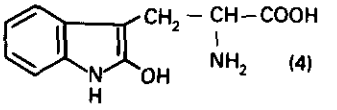
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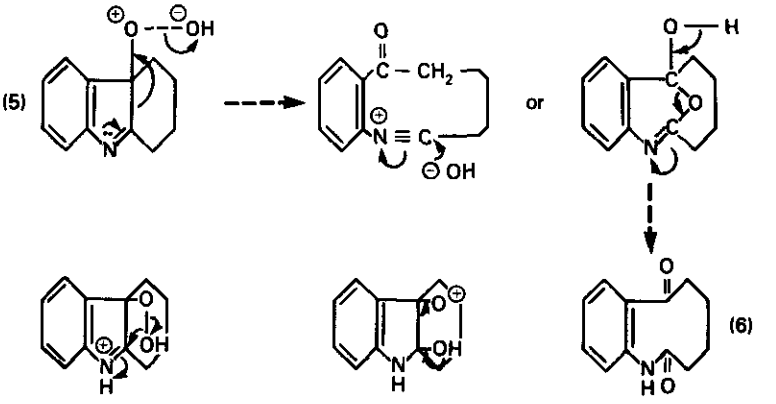
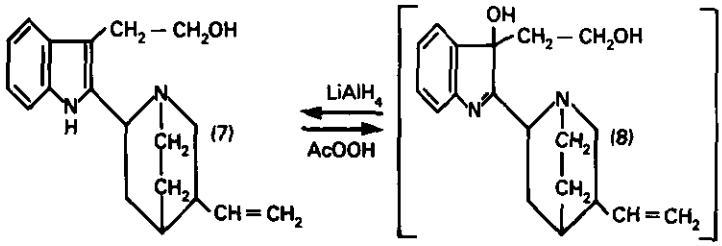
An aromatic substituent in the 2-position[6] permits the isolation of stable crystalline ozonides capable of showing ring-chain tautomerism.[7] The most artful tale raises little curiosity when it is known to be false: thus, neither the α-hydroxytryptophan, isolated from the hydrolysate of phalloidin,[8] is real,[9] nor its putative role as a prokynurenine in the pathway to ommochromes,[10] nor the simplistic picture I had when I studied the action of peroxyacids on indoles.[11]

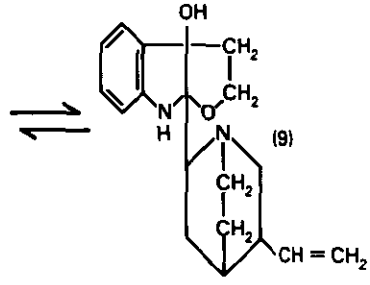
THE β-HYDRO(PERO)XYINDOLENINES [12]

The mechanism of oxidation of indoles fell into place when a model reaction, the (acid-

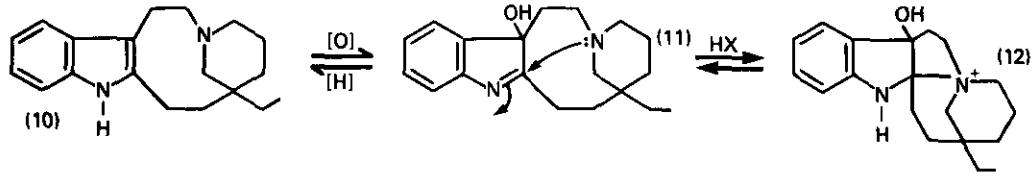

(5) or (6)

catalyzed) rearrangement of the hydroperoxide of tetrahydrocarbazole 5 → 6[13] was expanded to encompass the oxidative conversion of cinchonamine 7 to quinamine 9 via 7,[14] two alkaloids from





the cinchona bark. An analogous relationship connects quebrachamine 10 with rhazidigenine 11, in equilibrium with rhazidine 12[15] in the cell-sap of the plant.



Quebrachamine Rhazidigenine Rhazidine

Although this oxidation principle was formulated for tryptophan,[12] its experimental verification had to wait a quarter century: A valid model for the mechanism of oxidation of tryptophan to formylkynurenine - 25 years later,[16] was the title of a joint investigation made possible by the generosity and skill of my colleagues from Japan, Tohru Hino and Masako Nakagawa and their group.[16] Independently, Teruo Matsuura made notable contributions to this field. As W. E Savige had shown in 1975,[17] gentle conditions easily produce the elusive β-hydroxy-ψ-tryptophan 13 that cyclizes to an eseroline 14, predicted[12] and observed[18] with angular methyl before.

THE 2-HYDROXYINDOLES

With acid these intermediates dehydrate to 15 and hydrolyze to oxy- (16) and, in the presence of oxygen, dioxytryptophan (17), the subject of synthesis by, and a long exchange of letters with, my late friend Percy L. Julian.[19] Aqueous solutions of α-oxytryptophan (oxindolylalanine) on standing apparently autoxidize (via 17 or an equivalent) to kynurenine, probably the reason for

the initial postulation of the role of a prokynurenine for α-oxytryptophan.[20] Three colleagues[21-23] with whom I enjoyed cordial relations over many years, synthesized α-oxytryptophan or oxindolylalanine (16).

A letter from Munio Kotake, dated April 27, 1950, was the first sign from Japan after the war which, in charming English, informed me of his activities in this field:*

April 27, 1950

Dear Prof. Witkop:

I have received your sincere letter and three interesting reprints of your elaborate works which I have appreciated very much.

It is of special pleasure to me to remind that you had studied under Prof. H. Wieland, our mutual teacher.

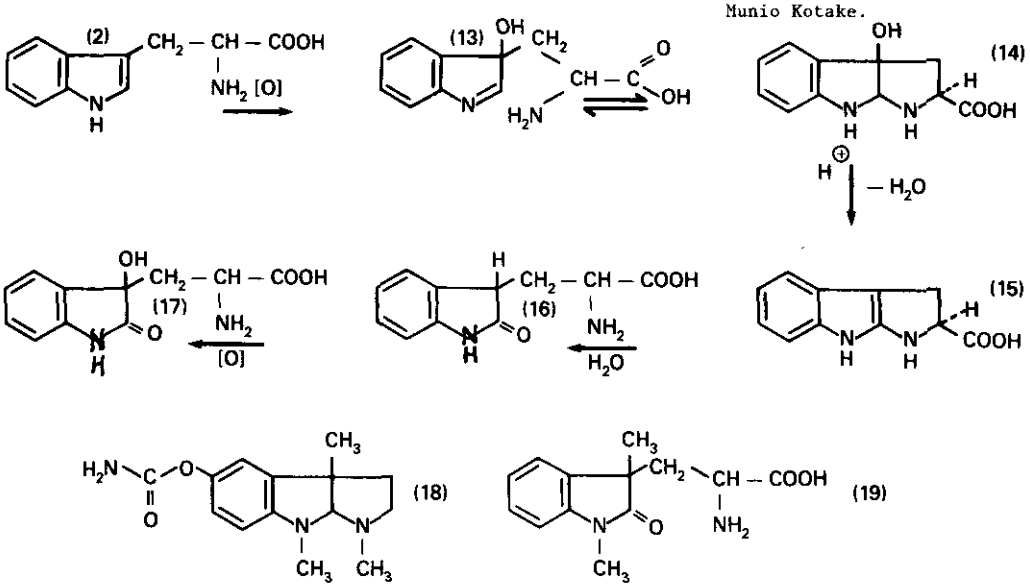
My friend of mind, Mr. Chikao Hondo, being the President of Mainichi Press, will leave for the United States on the 10th of May. I shall take advantage of this occasion to send you some of the samples of rac-hydroxy-tryptophane and rac-hydroxy-kynurenine, and also some new reprints of our recent works.

As we are conducting our researches under various difficulties, then my heart felt gratitude in due to your kind offers.

With full of thanks.

Very sincerely yours,

Munio Kotake.



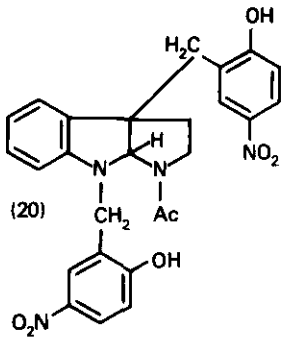
*The close contacts with Prof. Kotake proved to have important consequences, because they inspired the initiation of the NIH Visiting Program, inaugurated through the good offices of Profs. Kotake and Sakan who sent Siro Senoh to Bethesda where he entered my Laboratory on December 11, 1956, the first of more than 60 Visiting Scientists who subsequently came to my laboratory. In all several thousand young scientists from Japan have made the pilgrimage to NIH in the intervening years with most gratifying results for the international scientific community.

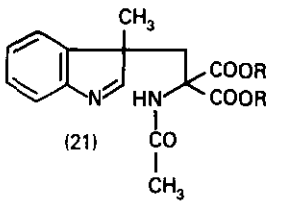
Kotake's synthesis of oxindolylalanine (1950)[21] precedes that of Percy Julian (1956)[23] and of Cornforth (1951)[22] although Julian whom as a student I admired for his first synthesis of physostigmine (18) in 1935[24], was able to construct 1,3-dimethyloxindolylalanine (19)[25] in the same year.

Although LSD forms an oxindole metabolite[26], oxindolylalanine, as Osamu Hayaishi tested in 1956, was not a metabolite of tryptophan or a substrate for tryptophan oxygenase (L-tryptophan: oxygen oxidoreductase (decyclizing) EC 1.13.11.11).

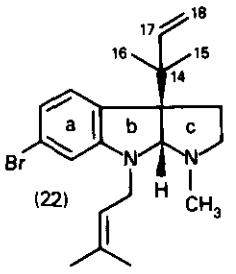
THE INTRAMOLECULAR CYCLIZATION OF INDOLAMINE DERIVATIVES

The tendency of the amino group in tryptophan or tryptamine to undergo intramolecular addition to tricyclic derivatives related to eseroline is noticeable in β-alkylation or

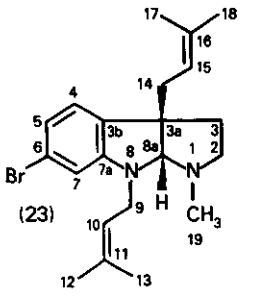




β-oxidation, two processes that lead to unstable indolenine intermediates. Such a β-alkylation is observed with 2-hydroxy-5-nitrobenzylbromide, the so-called Koshland reagent, to afford a dialkylated product, 20.[27] Even the acylated amino group is capable of adding to intermediate

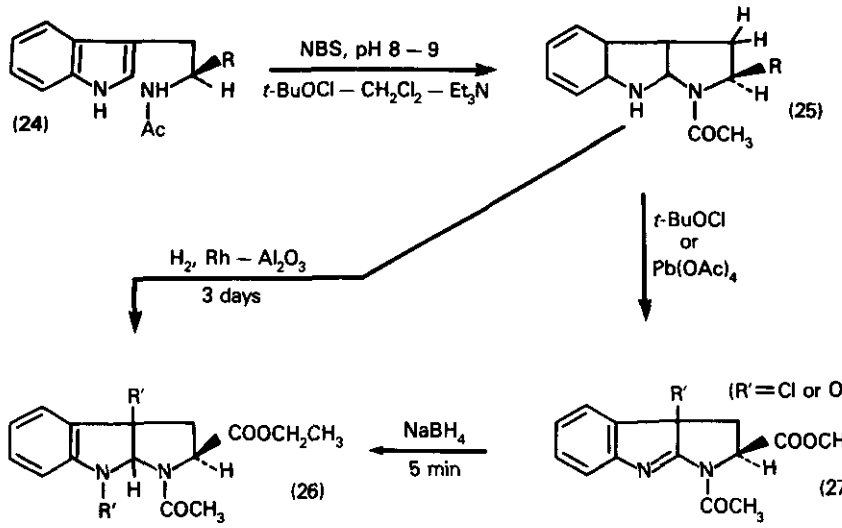


Flustramine A

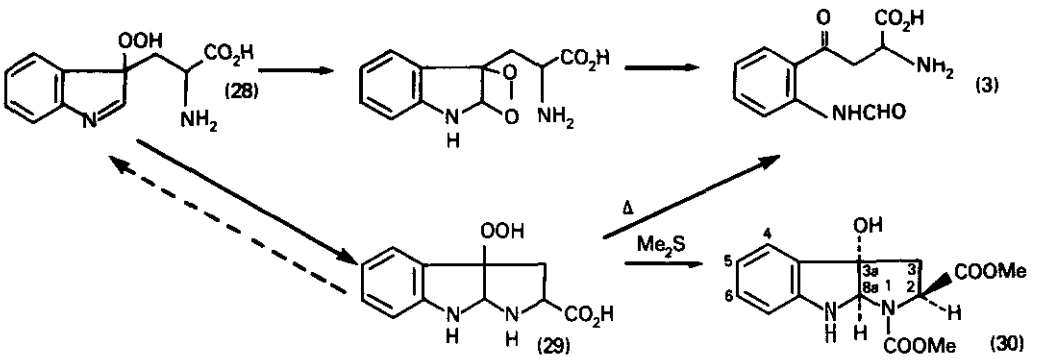


Flustramine B

indolenines preceding 20 or expressed in 21 [18] Flustramine A (22) and B (23) are recent examples of such alkylation products occurring in marine organisms.[28] The introduction of a β-chloro or β-hydroxy group is a theme with many variations (24-26)[29] which was explored with the help of Motonori Ōno.



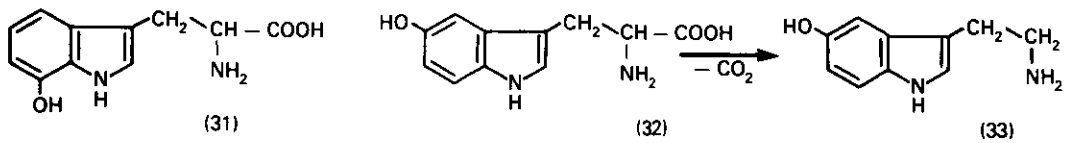
The tricyclic derivatives (29, cis and trans) of β-hydroperoxy-ψ-tryptophan (28)[17] are of some interest in another connection: I. P. Lapin has studied kynurenines as neuroactive tryptophan metabolites, especially their convulsive effects.[30-32] The tricyclic trans-3-hydroxytryptophan (30) on intracerebroventricular administration to albino mice produced some central



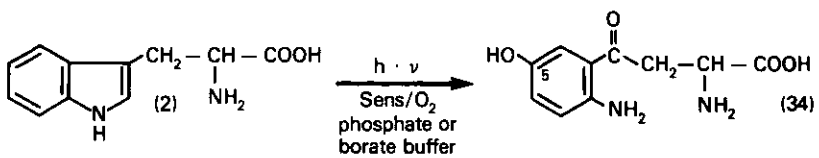
effects (ataxia, excitability) at 100 or 200 μg but not the kind of seizures that a ready in vivo conversion to kynurenine would be expected to produce (personal comm., Leningrad, May 12, 1981). 3-Hydroperoxyindole derivatives, on the other hand, inhibit a specific prostaglandin I_2 synthetase.[33]

5-HYDROXYTRYPTOPHAN AND THE NIH SHIFT

My first (Norwegian) doctoral student at Harvard, Arvid Ek, synthesized hydroxylated tryptophans, viz., 5- (32) and 7-hydroxytryptophan (31).[34] 5-Hydroxytryptophan (32) is a new naturally occurring amino acid, the precursor of serotonin, whose importance as a neurotransmitter, a precursor of melatonin, a regulator of sleep and affective disorders is still increasing.[14] A metabolite of 5-hydroxytryptophan in rabbit small intestine is 5-hydroxykynurenine (34)[36] which



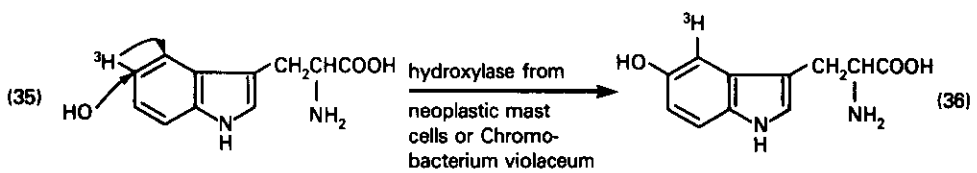
was first synthesized by Butenandt and his group.[37] In her last Christmas greetings (1982), Masako Nakagawa reported to me a one-step photosensitized oxygenation of tryptophan (2) leading



directly to 5-hydroxykynurenine (34)[37a]: Whether the aromatic hydroxylation involves an NIH-Shift or not, is under investigation.

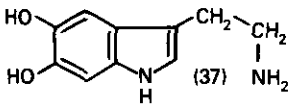
It was my good fortune to have Sidney Udenfriend next door at NIH who wasted no time to show in 1953 that L-5-hydroxytryptophan was the natural substrate of "aromatic amino acid decarboxylase."[38] He also aided in the development of a rapid spectrophotometric assay for L-amino acid oxidase based on L-kynurenine.[39]

Again, in collaboration with S. Udenfriend, a much more surprising phenomenon was discovered in 1966, namely, the migration of deuterium or tritium in 5^2H- of 5^3H-tryptophan (35) to



5-hydroxy-4^2H- or 4-^{3}H-tryptophan (27) with up to 90% retention of label.[40] This migration of a substituent in the process of enzymatic hydroxylation of aromatic substrates was termed "NIH-Shift"[41] and served as the starting point of worldwide investigations on the significance or danger of arene oxide intermediates, either in drug metabolism, long-range toxicity or carcinogenesis.[42-44] Here we observe "wie ein Tritt tausend Fäden regt" ("one step--and thousand threads arise . . ."). Selective exchange of nuclear protons in hydroxyindoles was just a parenthetic observation, mentioned here only to show the impact of a method still comparatively new at the time, viz., NMR spectroscopy.[45]

Although Arvid Ek did prepare 5,6-dibenzyloxytryptamine as early as 1953, it was not until Hans Schlosserger knew how to handle free 5,6- (or 5,7- (38) dihydroxytryptamine (37) that these



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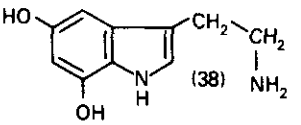

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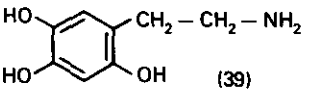
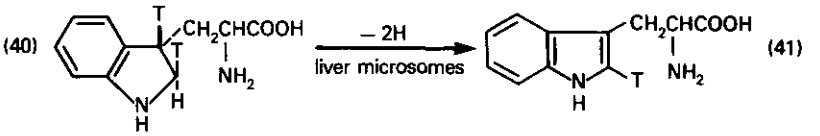


(39)

amines were discovered as serotinergic neurotoxins[46] in the same way as 6-hydroxy-dopamine (39) was first brought to life in a metabolic context, by my first scientific collaborator from Japan, Siro Senoh, when it turned out later to be an agent for chemical adrenectomy by mechanisms probably both involving related quinoid intermediates capable of addition of nucleophilic SH groups from adjacent (receptor) proteins (reviewed in ref. 46).

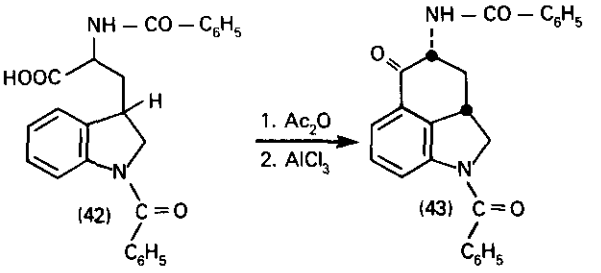
DIHYDROTRYPTOPHAN A SYNTHONE FOR LYSERSIC ACID

Both photoreduction[47] as well as reduction in the ground state[48] provided partially hydrogenated tryptophans, such as 2,3-dihydro- (or ditritio) L-tryptophan 40, convertible enzymatically



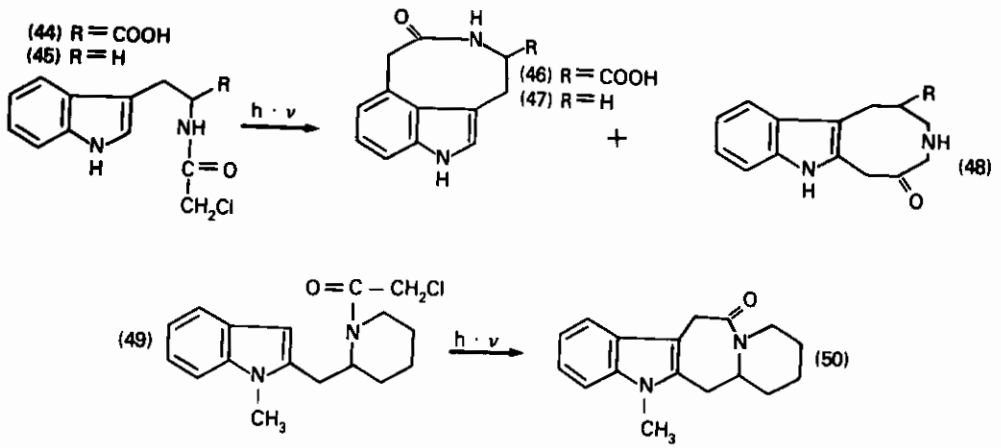
cis-[2,3-^{3}H] dihydro-L-tryptophan L-[2-^{3}H] tryptophan

to 2^{3}H-L-tryptophan (41). It's N,N'-dibenzoyl derivative 42 proved useful for intramolecular acylation involving the 4-position 43, a kind of functionalization with many uses, e.g., for synthetic access to lysergic acid derivatives[49] to unusual indole alkaloids, such as the regulovasines.[50]

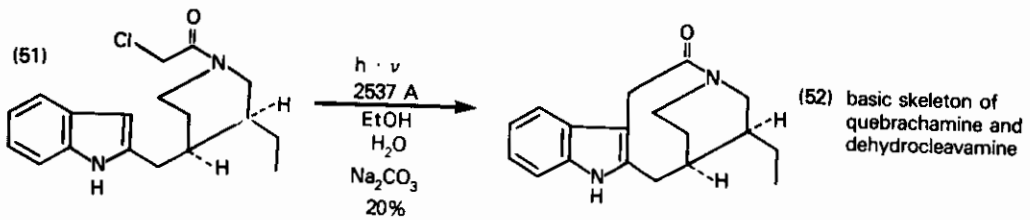


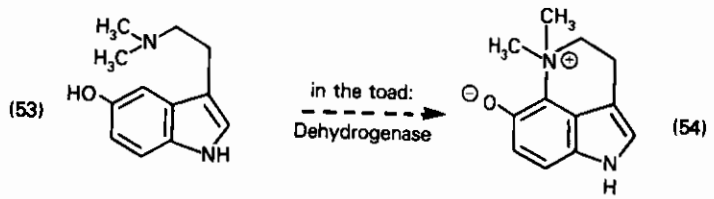
EXCIPLEXES INVOLVING THE 4-POSITION

A new type of photocyclization provides another approach to tricyclic indoles (46-48) involving the 4- (and 2-) position.[47] The starting materials are simple N-chloroacetyl-tryptophans (44), -tryptamines (45) or melatonins.[51] My colleague Osamu Yonemitsu has studied



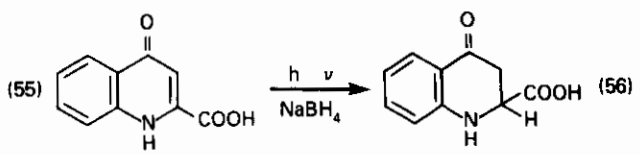
these competitive cyclizations into the 4- and 2- positions in considerable and sophisticated mechanistic detail.[52] The method has been expanded to photochemical modification of





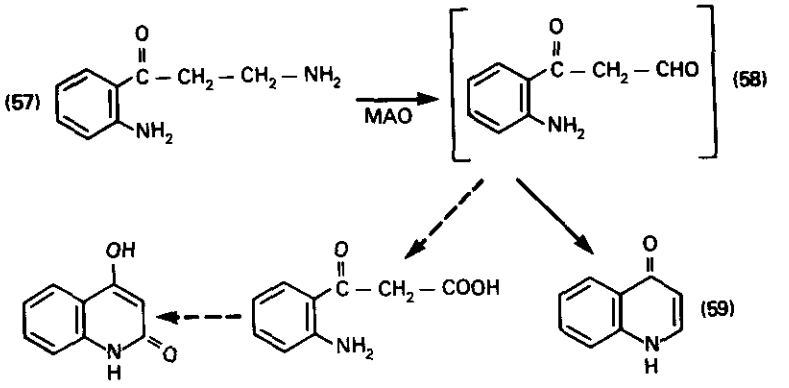
proteins in the presence of chloroacetamide[53,54] and to synthetic applications in the alkaloid field 49 → 52.[55,56] A natural product in which the 4-position is involved is dehydrobufotenin (54) which should arise in the toad from bufotenin (53) by an enzyme in toad glands that Siro Senoh attempted in vain to isolate more than 20 years ago.

Photoreduction, in the hands of Takashi Tokuyama, proved useful with other tryptophan metabolites and transformed kynurenic acid (55) to the so-called kynurenine yellow (56).[57]

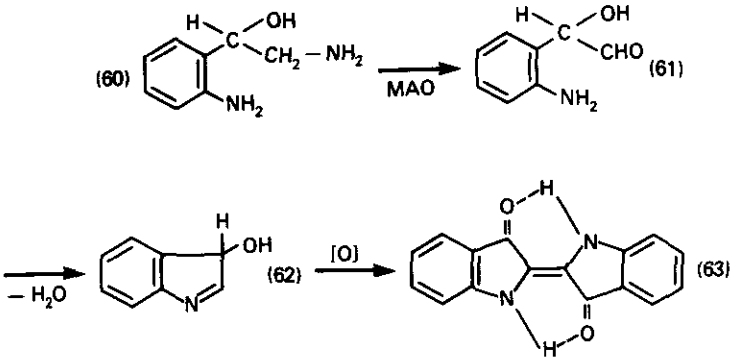


(NOR) KYNURAMINE AND RAPID MAO ASSAYS

The "biogenic amine" corresponding to kynurenine was termed kynuramine 57; it is accessible by ozonolysis of N-carbobenzoxytryptamine and subsequent hydrogenolytic debenzylation.[58] The disappearance of λ_{max} 360 nm of kynuramine 57 on incubation with monamine oxidase and formation of γ-quinolone (59) via the intermediate aldehyde 58 was introduced as a rapid spectrophotometric assay for this conspicuous enzyme. The appearance of λ_{max} 600 nm of indigo 63 via (61) and (62),



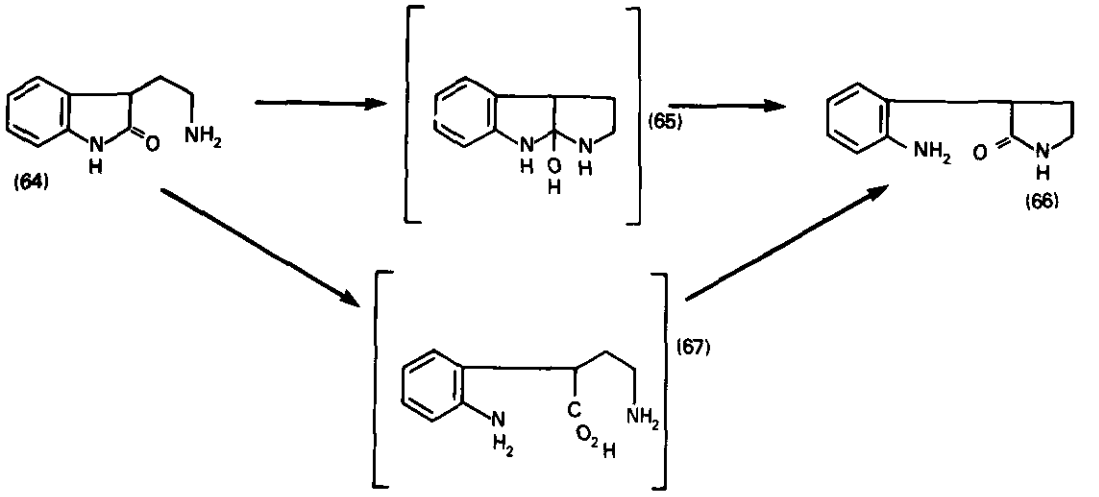
is observed when dihydronorkynuramine 60, not a good substrate, is incubated with monoamine



oxidase,[59] a reaction that, understandably, competes neither with the natural, nor the industrial synthesis of, indigo.

RING-CHAIN TAUTOMERISMS OF 2-HYDROXYTRYPTAMINE

Oxindole analogs of tryptamine and serotonin attracted attention as potential inhibitors of the enzymes involved in the biosynthesis and metabolic breakdown of serotonin,[60] but opened a Pandora's box of ring-chain tautomers, suspected at the time (1957), but not successfully isolated until, almost 20 years later, Nakagawa, Hino and their group explained the reactivity of oxytryptamine 64, in terms of its easy conversion to 3-(o-aminophenyl)-2-pyrrolidone 66,



either via 65 or 67. Many more compounds are formed when oxygen is not excluded.[61]

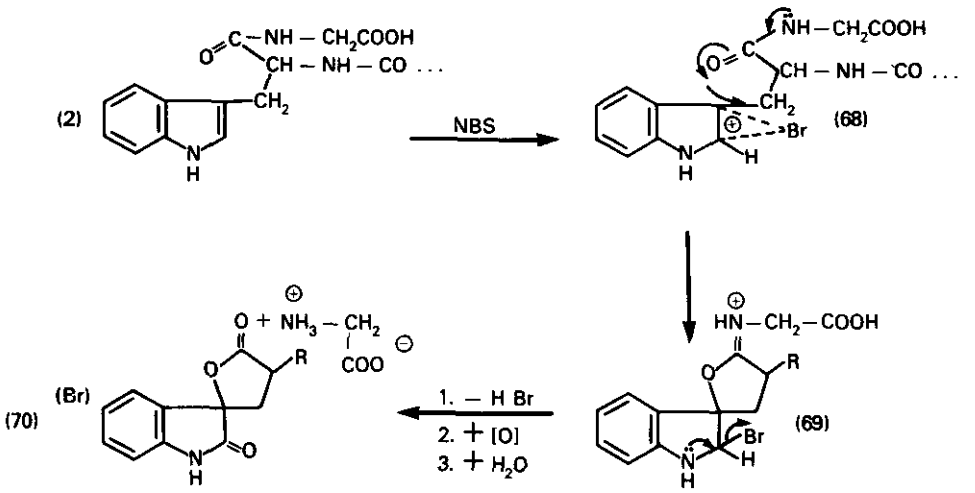
SELECTIVE CLEAVAGE OF THE TRYPTOPHYL PEPTIDE BOND

Tryptophan (2), as such, in peptides or bound in proteins, reacts so fast (68 → 69 → 70) with positive halogen, e.g., N-bromosuccinimide, tribomocresole or 2-nitrophenylsulfonyl chlorides (NPSCl, DNPS-Cl, Scoffone, Fontana, 1966) that these reactions permit not only an easy titration of bound tryptophan[62,63] but also selective cleavage of tryptophyl peptide bonds, under appropriate conditions. This cleavage has been utilized for sequencing of peptides and proteins, but not as systematically and extensively as the cyanogen bromide cleavage, discovered with the late Erhard Gross in 1961.[64]

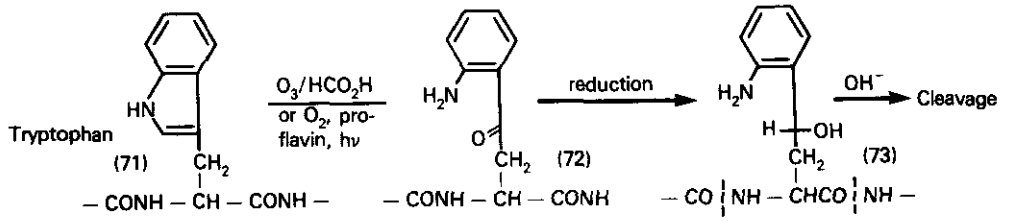
The method was used to probe the sequence of gramicidin A the most lipophilic naturally occurring peptide.[65] In a sequence of 15 amino acids it contains 4 tryptophan residues which liberate the subsequent leucine or the terminal ethanolamine in modest yields (ref. 63, p. 160).

As a unique former of channels in artificial membranes gramicidin A, despite the knowledge of its primary sequence, has so far not betrayed the secret of its way to transport ions. Of the four tryptophans present the carbonyls of Tryp-11, 13 and 15 are directed outward into the aqueous medium in the postulated model of the trans-membrane channel. The current model calls for a single-stranded β-helix, head to head dimerization and a left-handed helix. But the search for monovalent cation binding sites in the gramicidin channel continues.[66] The last word will probably be spoken by the Roentgen-ray crystallographer.[67]

The classical study of kynurenine by Butenandt[3] reports the formation of an (N,N'-diacetyl)-lactone from dihydrokynurenine. Almost one generation later this reaction found a new use:

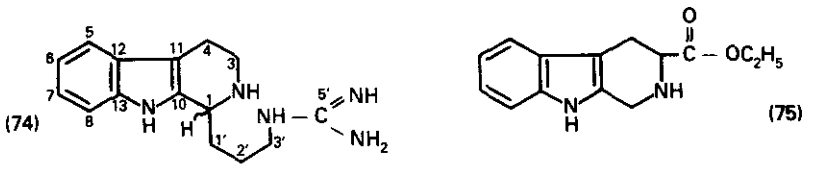


ozonolysis of bound tryptophan (71), reduction (73) of bound kynurenine (72) so formed and utilization of the new neighboring γ-hydroxyl group, in the hands of Fumio Sakiyama and his group, has made possible useful cleavages, probably more preferential than selective (reviewed in ref. 63).



NEW IMPORTANCE OF TETRAHYDROHARMANES

M. Ozaki helped to develop tetrahydroharmanes as inhibitors of monoamineoxidase.[68] Very recently such condensation products 75 of tryptophan have attracted attention as -- probably only exogenous --inhibitors of benzodiazepine receptors,[69] suitable as models for anxiety in primates,[70] and as (-)-trypargine (74), a serotinergic principle, isolated from the African frog Kassina senegalensis.[71,72]



(−) - trypargine: C (1) - αH

OXIDATION OF THE TRYPTOPHAN SIDE CHAIN

The mobile π-electron system of tryptophan is involved in activating all seven positions of the indole nucleus for the attack of nucleophilic oxidants and for radical or radical-ion reactions, be they inter- or intramolecular. In order to engage the "benzylic" position in the side chain, an oxidant must have two properties: on the one hand it must not have nucleophilic character, on the other hand, it must tie down the mobile π-electron system, preferentially by forming a π-complex, often easily recognized by intense color phenomena. Here the chronicler becomes a delighted spectator and the memories become "notes of a tryptofun watcher". Osamu Yonemitsu achieved selective side chain oxidation of indoles by the use of dichlorodicyanobenzoquinone (DDQ)[73] which is known to oxidize benzylic methylene groups, e. g., tetralins to tetralones.[74] This reaction in the hands of Takashi Tokuyama, helped to prepare the keto-precursor of erythro- and threo-β-hydroxy-N-acetyltryptophanamide,[75] of which the former is formed by tryptophan side chain oxidase (or "indolyl-3-alkane-α-hydroxylase"[76]), an enzyme discovered by Osamu Hayaishi in Pseudomonas species.[77] One of the products is N-acetyl-α,β-didehydrotryptophanamide belonging to the interesting class of dehydropeptides. The tryptophan side chain oxidase probably belongs to a newly discovered group of enzymes, the so-called quinoproteins which contain a pyrroloquinoline quinone (PQQ) as their coenzyme.[78-80] Such an enzyme system would qualify for the formation of π-complexes as indicated by the model oxidations with DDQ.

EPILOG

As informative as all these model reactions are, they only point the way to the dynamics of tryptophan metabolites. Their involvement in sleep patterns, psychic disorders, moods and depressions, is an area under active pharmacological and clinical investigation.[35]

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REFERENCES

1. P. W. Hickmott, Enamines: Recent Advances in Synthetic, Spectroscopic and Stereochemical Aspects, Tetrahedron 18, 1975-2050 (1982).
2. Y. Kotake, Ergebnisse der Physiologie, 37, 245 (1935).
3. A. Butenandt, W. Weidel, E. Becker, V. Derjugin, Hoppe-Seyler's Z. für physiol. Chemie 279, 27 (1943).
4. Adolf Butenandt, Das Werk eines Lebens, herausg. v.d. Max Planck Ges. Band 1, 1-32. Vandenhoeck & Ruprecht Göttinger und Zürich (1981).
5. B. Witkop, Ann. 556, 103 (1944).

6. H. Kissman and B. Witkop, The use of various aminomalonates in the synthesis of α-substututed tryptaphones. J. Am. Chem. Soc. 75, 1967-1974, 1953.
7. B. Witkop, J. B. Patrick and H. Kissman, Chem. Ber. 85, 949-977.
8. H. Wieland and B. Witkop, Ann. 543, 171 (1940).
9. Th. Wieland, Chemie in unserer Zeit, 13, 56 (1979).
10. A. Butenandt, W. Weidel, E. Becker, Naturwiss. 28, 447 (1940).
11. B. Witkop, Ann. 558, 98 (1947).
12. A. Ek. H. Kissman, J. B. Patrick and B. Witkop, Experientia 8, 36 (1952).
13. B. Witkop and J. B. Patrick, J. Am. Chem. Soc. 73, 2196 (1951); the differentiation between the two possible pathways in this Rearrangement, viz., Criegee and/or dioxetane mechanisms, became possible much later: S. Muto and J. C. Bruice, J. Am. Chem. Soc. 102, 1371 (1980).
14. B. Witkop, J. Am. Chem. Soc. 72, 2311 (1950).
15. S. Markey, K. Biemann and B. Witkop, Tetrahedron Letters 157 (1967).
16. M. Nakagawa, H. Watanabe, S. Kodato, H. Okajima, T. Hino, J. L. Flippen, B. Witkop, Proc. Nat. Acad. Sci. USA 74, 4730 (1977); M. Nakagawa, S. Kato, S. Kataoka, S. Kodato, H. Watanabe, H. Okajima, T. Hino and B. Witkop, Chem. Pharm. Bull. 29, 1013 (1981).
17. W. E. Savige, Aust. J. Chem. 28, 2275 (1975).
18. B. Witkop and R. K. Hill, J. Am. Chem. Soc. 77, 6592 (1955).
19. B. Witkop: Percy Lavon Julian (1899-1975), Biographical Memoirs, Vol. 52, p. 223, Nat. Acad. Sciences, Washington, D. C. 1980.
20. A. Butenandt, H. Hellmann and G. Hauser, Hoppe-Seyler's Z. Physiol. Chem. 289, 225 (1952).
21. M. Kotake, T. Sakan and T. Miwa, J. Am. Chem. Soc. 72, 5085 (1950).
22. J. W. Cornforth, R. H. Cornforth, C. E. Dalgliesh and A. Neuberger, Biochem. J. 48, 591 (1951).
23. P. L. Julian, E. E. Dailey, H. C. Printy, H. L. Cohen and S. Hamashige, J. Am. Chem. Soc. 78, 3503 (1956).
24. P. L. Julian and J. Pikl, J. Am. Chem. Soc. 57, 755 (1935).
25. P. L. Julian, J. Pikl and F. E. Wantz, J. Am. Chem. Soc. 57, 2026 (1935).
26. K. Freter, J. Axelrod and B. Witkop, J. Am. Chem. Soc. 79, 3191 (1957).
27. T. F. Spande, M. Wilchek and B. Witkop, J. Am. Chem. Soc. 90, 3256 (1968).
28. J. S. Carlé and C. Christophersen, J. Org. Chem. 45, 1586 (1980).
29. M. Ohno, T. F. Spande and B. Witkop, J. Am. Chem. Soc. 90, 6521 (1968): 92, 343 (1970).
30. I. P. Lapin, Trends in Pharmacol. Sci. 410 (1980).

31. I. P. Lapin, Pharm. Biochem. and Behavior 14, 589 (1981).

32. I. P. Lapin, Epilepsia 22, 257 (1981).

33. Z. Terashita, K. Nishikawa, S. Terao, M. Nakagawa, E. T. Hino, Biochem. Biophys. Res. Comm. 91, 72 (1979).

34. A. Ek and B. Witkop, J. Am. Chem. Soc. 75, 500 (1953).

35. D. A. Bender, Molec. Aspects of Medicine, Biochemistry of Tryptophan in Health and Disease, 6, 101-197 (1983).

36. M. Fujiwara, M. Shibata, Y. Nomiyama, T. Sugimoto, F. Hirata, T. Tokuyama, S. Senoh and O. Hayaishi, Proc. Natl. Acad. Sci. USA 76, 1145, (1979).

37. A. Butenandt, G. Schulz and G. Hanser, Hoppe-Seylers Z. Physiol. Chem. 295, 404 (1953).

37a. M. Nakagawa, Y. Yokoyama, S. Kato and T. Hino, The 103th Annual Meeting of the Pharmaceutical Society of Japan, Abstracts of Papers, p. 112, Tokyo, April (1983).

38. C. T. Clark, H. Weissbach and S. Udenfriend, J. Biol. Chem. 210, 139 (1954).

39. H. H. Weissbach, A. V. Robertson, B. Witkop and S. Udenfriend, Anal. Biochem. 1, 286 (1960).

40. J. Renson, J. Daly, H. Weissbach, B. Witkop and S. Udenfriend, Biochem. Biophys. Res. Com. 5, 504 (1966).

41. G. Guroff, J. W. Daly, D. Jerina, J. Renson, S. Udenfriend and B. Witkop, Science 158, 1524 (1967).

42. B. Witkop and Y. Kanaoka, The NIH-Shift, Kagaku no Ryōiki 23, 37 (1969), in Japanese.

43. B. Witkop, Experientia 27, 1121 (1921).

44. B. Witkop, The NIH Shift and its implications for the Mechanism of Biological Oxidations, Current Topics in Biochemistry, 109-133, Academic Press, New York, 1973.

45. J. W. Daly and B. Witkop, J. Am. Chem. Soc. 89, 1032 (1967).

46. H. G. Baumgarten, S. Jenner, A. Björklund, H. P. Klemm and H. G. Schlosserger, Biology of Serotonergic Transmission, Editor N. N. Osborne, pp. 250, John Wiley, New York 1982.

47. O. Yonemitsu, P. Cerutti and B. Witkop, J. Am. Chem. Soc. 88, 3941 (1966).

48. J. W. Daly, A. B. Mauger, O. Yonemitsu, V. K. Antonov, K. Takase and B. Witkop, Biochemistry, 6, 648 (1967).

49. J. Rebek, Jr. and D. F. Tai, Tetrahedron Letters 24, 859 (1983).

50. J. Rebek and Y. K. Shue, J. Am. Chem. Soc. 102, 5426 (1980).

51. T. Kobayashi, Jr., T. F. Spande, T. F. Aoyagi and B. Witkop, J. Med. Chem. 12, 636 (1969).

52. O. Yonemitsu: Electron Transfer Photochemistry, Yakugaku Zasshi 102, 716-734 (1982).

53. S. Naruto and O. Yonemitsu, Tetrahedron Letters 3399 (1975).

54. T. Hamada and O. Yonemitsu, Chem. Pharm. Bull. 25, 271 (1977).

55. R. J. Sundberg and F. X. Smith, J. Org. Chem. 40, 2613 (1975).

56. A. Wu and V. Snieckus, Tetrahedron Letters 2060 (1975).

57. T. Tokuyama, S. Senoh, T. Sakan, K. S. Brown, Jr., and B. Witkop, J. Am. Chem. Soc. 89, 1017 (1967).

58. H. Weissbach, T. E. Smith, J. W. Daly, B. Witkop and S. Udenfriend, J. Biol. Chem. 235, 1160 (1960).

59. Y. Kanaoka, H. Weissbach, T. E. Smith and B. Witkop, J. Am. Chem. Soc. 83, 732 (1961).

60. K. Freter, H. Weissbach, B. Redfield, S. Udenfriend and B. Witkop, J. Am. Chem. Soc. 80, 983 (1958).

61. M. Nakagawa, T. Maruyama, K. Hirakoso and T. Hino, Tetrahedron Letters 21, 4839 (1980).

62. B. Witkop, Nonenzymatic Methods for the Preferential and Selective Cleavage and Modification of Proteins, Adv. Protein Chemistry 16, 221 (1961).

63. T. F. Spande, B. Witkop, Y. Degani and A. Patchornik, Adv. Protein Chem. 24, 98 (1970).

64. E. Gross and B. Witkop, J. Am. Chem. Soc. 83, 1510 (1961).

65. R. Sarges and B. Witkop, J. Am. Chem. Soc. 86, 1862 (1964); 87, 2011 (1965).

66. D. W. Urry, K. U. Prasad and T. L. Trapane, Proc. Nat. Acad. Sci. 79, 390 (1982).

67. For the last ten years Isabella L. Karle of the Naval Research Laboratory, Washington, D. C., has -on and off- evaluated limited diffraction data from crystalline gramicidin A and at times seen a temporary lifting of the veil.

68. M. Ozaki, H. Weissbach, A. Ozaki, B. Witkop and S. Udenfriend, J. Med. Pharm. Chem. 2, 591 (1960).

69. C. Braestrup, M Nielson and C. E. Olsen, Proc. Nat. Acad. Sci. 77, 2288 (1980).

70. P. Ninan, T. Insel, J. Cook, P. Skolnick and S. Paul, Science 218, 1333 (1982).

71. Synthesis: M. Shimizu, M. Ishikawa, Y. Komoda, T. Nahajima, Chem. Pharm. Bull. 30, 909 (1982).

72 B. Witkop, Amphibian Venoms, The Alkaloids, XXI, ed. A. Brossi, pp. 139-253, Acad. Press, New York, 1983.

73. Y. Oikawa and O. Yonemitsu, Selective oxidation of the side chain at C-3 of indoles, J. Org. Chem. 42, No. 7 1213 (1977).

74. H. D. Becker, J. Org. Chem. 30 982 (1965); J. W. A. Findlay and A. B. Turner, J. Chem. Soc. C, 23 (1971).

75. I. Karle, Int. J. Peptide Protein Res. 16, 471-476 (1980).

76. J. Roberts and H. J. Rosenfeld, J. Biol. Chem. 252, 2648-2656 (1977).

77. H. Ushiro, K. Takai, Y. Noda, S. Narumiya, T. Tokuyama and O. Hayaishi, Tryptophan side chain oxidase from pseudomonas, J. Biol. Chem. 253, 9002-9008 (1978).

78. J. A. Duine and J. F. Jzn., Trends Biochem. Sci. 6, 278-280 (1981).

79. R. de Beer, J. A. Duine, J. Frank and J. Westerling, Eur. J. Biochem. in press (1983).

80. W. van der Graff, J. A. Duine, J. Frank and J. A. Jongejan, Tryptophan side chain oxidase from pseudomonas: a novel quinoprotein? Fourth International Meeting on Tryptophan Metabolism Biochemistry, Pathology and Regulation, Max-Planck-Institut für Biochemie, Martinsried, April 19-22, 1983, Abstracts p. B30.

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