RESEARCH ON BIOACTIVE NATURAL PRODUCTS AT THE NIH (1976-1988)

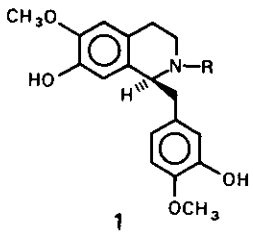
Arnold Brossi

Medicinal Chemistry Section, Laboratory of Analytical Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, U. S. A.

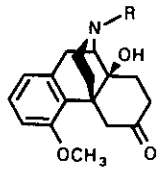
Isoquinolines continued to play a major role in my activities at the National Institutes of Health in Bethesda (NIH), after I joined Bernhard Witkop's Laboratory of Chemistry in the Fall of 1976. A practical synthesis of (±)-norreticuline and its optical isomers (1) was soon accomplished[1] by taking advantage of acetonitrile as a superior solvent in Bischler-Napieralski cyclizations of unprotected phenolic intermediates[2]. The finding that S-reticuline derives from S-norcoclaurine[3], and converts into the R-isomer by an oxido-reduction process[4] will further stimulate research in this classical field of alkaloids with focus on mammalian opium alkaloids[5]. Exploration of structure-activity relationships of synthetic morphinans revealed that 4-methoxy substituted ketomorphinans such as 2[6] and dihydrodeoxymorphine (3)[7], represented structures of unexpected potent antinociceptive agents. The study of mammalian alkaloids remained a subject of continued interest[8]. The isoquinoline-1-carboxylic acid 4, recently obtained in optically active forms[9], is methylated by O-methyltransferase *in vitro* exclusively in the 7-OH group; the S-isomer is methylated at a rate 6-times faster than the R-isomer[10]. Carboxylic acid 4 decomposes at physiological pH to the yellow quinone methine 5 which undergoes further decomposition on air oxidation. The alkaloid weberine (6, R^1=H,R^2=CH_3) of the rare class of tetramethoxy substituted TIQ obtained by synthesis[11] is now explored together with Creed Abell, School of Pharmacy, University of Texas, Austin, for enzyme inhibition. Witkop's research on histrionicotoxin from Columbian arrow poison frogs showed this compound and its hydrogenated congeners to be useful tools to study ion translocation in the nicotinic acetylcholine receptor[12]. Both optical isomers of perhydrohistrionicotoxin were prepared[13], as well as the desamyl- and desamyldesbutyl (7) analogs[14,15]. It was interesting to learn from Edson X. Albuquerque at the School of Medicine, University of Maryland, *Baltimore, with whom we collaborated*, that the optical isomers did not greatly differ in their actions on the nicotinic acetylcholine receptor-ion channel complex, in contrast to the nicotinic agonist D and L - anatoxin a for which new synthetic approaches were studied.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which depletes dopamine neurons of the substantia niagra and develops Parkinsonian syndromes in humans, has to undergo metabolic conversion into $MPDP^+$ (8) and fully aromatic MPP^+[16]. Synthesis of 8 from the N-oxide of MPTP[17] and that of many analogs of MPTP was reported[18]. The structure requirements for neurotoxicity of MPTP were found to be unusually rigid[19]. We profited again from an excellent collaboration with Creed Abell and his colleagues at the University of Texas in Austin. The Chinese antimalarial artemisinin (9) and some of its derivatives is being investigated with Chinese scientists working at the NIH. Pyrrolidine alkaloids represented by the trans-isomer 10 occur in ant species and were prepared from the Lukes-Sorm dilactam[20]. It was found that (±)-10 was a potent vasodilator and that the allergic reactions noted in man after stinging may well be connected with the presence of such alkaloids[21].

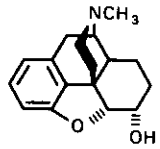
Hormothamnione (11) from blue-green algae, reported to have antiviral properties, was prepared by total synthesis but found to have no effect on the growth of HIV virus[22]. Continuation of our efforts to modify structures of known antimalarial drugs focused on the Chinese antimalarial qinghaosu and its derived arteether which are both potent blood schizontocides of a new kind[23]. This collaboration with WHO and Walter Reed, through Craig Canfield, continued to be fruitful and efficient. Exploring retinoids with Michael Sporn at the National Cancer Institute led to 2-retinylidine-1,3-diketones such as 12 which act as prodrugs of retinal[24]. Dihydrofluoresceine diacetate (DADF) was found useful to characterize alcohols and amines as esters and amides, respectively: after exposure to ammonia and iodine vapors on TLC-plates they formed deep red dyes which can be detected in nanomolar quantities by uv- and fluorescense techniques[25]. Whereas much of the progress discussed above followed thoughts and ideas nourished by earlier work, the two major topics covering my activity at the NIH during the last couple of years, physostigmine (14) and colchicine (15), were chosen by intent and purpose. Physostigmine (14), extensively studied by Polonovski in France around 1920 and prepared by total synthesis by Julian[26], has interesting anti-cholinesterase activity and is centrally more active than synthetic quaternary analogs, such as pyridostigmine and neostigmine. Variation of the carbamate side chain in (14)[27] and at N(1)[28], obtained by an improved synthesis of eseroline from Phy[29] with new resolution techniques[30], showed interesting activities against AChR and BChR. (-)-N(1)-Norphysostigmine emerged from these studies as a potent compound and is now being further investigated. Colchicine (15), a classical drug, has potent antimitotic effects and is an antiinflammatory, but too toxic in man to be clinically useful in arthritis[31]. Also, its present clinical uses against gout and Familial Mediterranean



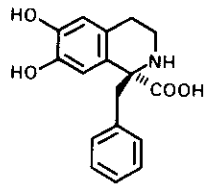
1



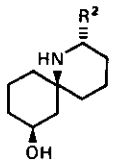
2



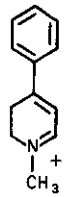
3



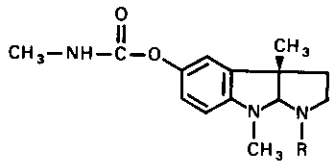
4



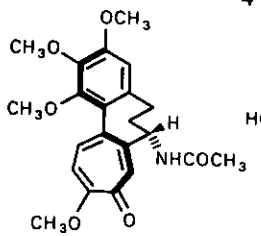
7



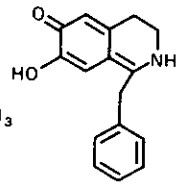
8



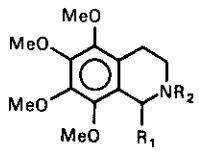
14



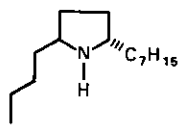
15



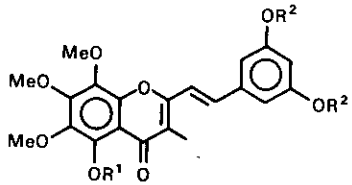
5



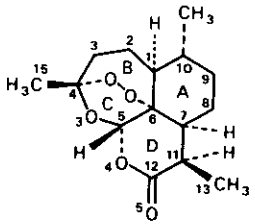
6



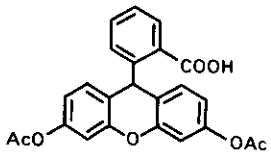
10



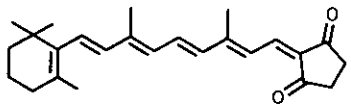
11



9



13



12

Fever (FMF) could benefit from the finding of less toxic analogs. With the knowledge that colchicine binds to tubulin and that affinity parallels antitumor effect the stage was set to test a large series of analogs of colchicine[32], culminating in the finding of the antitumor compound 3-demethylthiocol- chicine and the antiinflammatory agent (-)-2,3-didemethylcolchicine[33]. It was found that (-)-, (+)-, and (±)-colchicine and deacetamidocolchicine in the presence of tubulin form complexes displaying molecular asymmetry. Natural colchicine was determined to be (aS,7S)-colchicine[34]. This finding elaborated in collaboration with Herman Yeh from our Institute has far reaching consequences and is now further explored with synthetic tetramethoxybiphenyls.

If one looks for the guiding ideas in my work, done at Roche earlier and over the last 10 years at the NIH, one could conclude that its focus lies on and its application to enantiospecificity of drug action and its application to natural products. Most compounds were prepared and studied in the form of both optical isomers (emetine, griseofulvin, quinine, apomorphine, morphine, colchicine, physostigmine, isoquinoline alkaloids of mammalian origin, perhydrohistrionicotoxin etc.). This not only led to the recognition that most of them excerted their biological effects as a single enantiomer, with the notable exeptions of quinine and perhydrohistrionicotoxin, which in the systems studied were equally effective agents when tested as (-)- and (+)-enantiomers, and the discovery that unnatural (+)-physostigmine, which has practically no anticholinesterase activity, is an effective antidote to nerve gas poisons in experimental animals. This then leads to the conclusion that medicinal chemists are well advised to prepare their target compounds displaying atomic and molecular asymmetry in the form of both optical isomers. There is now ample methodology available to ensure effective and practical optical resolution, so that economic considerations can no longer be accepted as an excuse for not separating biologically active racemates. The largely unknown long-term effects of biologically inert isomers present in racemic mixtures, which may only be recognized in man after long exposure, will undoubtedly accelerate the design of optically active drugs. It is hoped that our exercises accomplished with a team of able colleagues from many countries, together with colleagues from industry and academia will have helped in the development of optically active drugs.

The National Institutes of Health in Bethesda, Maryland, is a delightful place to do research. It provides a stimulating atmosphere which is addictive and keeps scientists interested in their work. The fact that one can choose ones own research topics and the professional collaborators who come from all over the world is exiting, and differs from

my experience in industry where these matters often were decided by administrators who often made things difficult and complicated. A place like the NIH, naturally attracts all kind of people, some think very highly of themselves. This clearly did not bother me as long as they were well mannered and colleagal.

REFERENCES

1. K. C. Rice and A. Brossi, J. Org. Chem., 1980, 45, 592.
2. S. Teitel and A. Brossi, J. Heterocyclic Chem., 1968, 5 825.
3. R. Stadler, T. M. Kutchan, S. Loeffler, N. Nagakura, B. Cassels, and M. H. Zenk, Tetrahedron Lett., 1987, 28, 1251.
4. E. Brochman-Hanssen, "The Chemistry and Biology of Isoquinoline Alkaloids", (J. D. Phillipson, M. F. Roberts and M. H. Zenk, eds.). Springer-Verlag, New York, 1985, 229-239.
5. C. J. Weitz, K. F. Faull, and A. Goldstein, Nature, 1987, 330, 674.
6. H. Schmidhammer, A. E. Jacobson, and A. Brossi, Med. Res. Reviews, 1983, 3, 1.
7. J. Reden, M. F. Reich, K. C. Rice, A. E. Jacobson, A. Brossi, R. A. Streaty and W. A. Klee, J. Med. Chem., 1979, 22, 256.
8. A. Brossi, Heterocycles, 1978, 11, 521; M. A. Collins, "The Alkaloids", Vol 21 (A. Brossi, ed.), Academic Press, New York, 1983, 329-358; G. Brigmann, Nachr. Chem. Tech. Lab. (West Germany), 1986, 34, 222.
9. M. Chrzanowska, B. Schönenberger, A. Brossi, and J. Flippen-Anderson, Helv. Chim. Acta, 1987, 70, 1721.
10. M. D. Rozwadowska, M. Chrzanowska, A. Brossi, C. R. Creveling, M. B. Bembenek, and C. W. Abell, ibid, submitted.
11. K. Takahashi and A. Brossi, Heterocycles, 1982, 19, 691.
12. B. Witkop and E. Gössinger, "The Alkaloids", Vol. 21, (A. Brossi, ed.), Academic Press, New York, 1983, 139-253.
13. K. Takahashi, B. Witkop, A. Brossi, M. A. Maleque, and E. X. Albuquerque, Helv. Chim. Acta, 1982, 65, 252.
14. K. Takahashi, A. E. Jacobson, C. P. Mak, B. Witkop, A. Brossi, E. X. Albuquerque, J. E. Warnick, M. A. Maleque, A. Bavoso, and J. V. Silverton, J. Med. Chem. 1982, 25, 919.

15. W. Gessner, K. Takahashi, B. Witkop, A. Brossi, and E. X. Albuquerque, Helv. Chim. Acta, 1985, 68, 49.

16. W. Gessner, A. Brossi, R. Shen, and C. W. Abell, FEBS Lett., 1985, 183, 345.

17. W. Gessner, A. Brossi, R. Shen, R. R. Fritz, and C. W. Abell, Helv. Chim. Acta, 1984, 67, 2037.

18. W. Gessner, A. Brossi, R. Shen, and C. W. Abell, J. Med. Chem., 1985, 28, 311.

19. W. Gessner, A. Brossi, R. R. Fritz, N. T. Patel, and C. W. Abell, "MPTP:A Neurotoxin Producing A Parkinsonian Syndrome", Academic Press, New York, 1986, 557-561; A. Brossi, W. Gessner, R. R. Fritz, M. E. Bembeneck, and C. W. Abell, J. Med. Chem. 1986, 29, 444.

20. R. Alonso, W. Gessner, K. Takahashi, and A. Brossi, Syn. Commun., 1988, 18, 37.

21. W. Gessner, K. Takahashi, A. Brossi, M. Kowalski, and M. A. Kaliner, Helv. Chim. Acta, 1987, 70, 2003.

22. R. Alonso and A. Brossi, Tetrahedron Lett., 1988, 29, 735.

23. A. Brossi, B. Venugopalan, L. Dominguez Gerpe, H. J. C. Yeh, J. L. Flippen-Anderson, P. Buchs, X. D. Luo, W. Milhous, and W. Peters, J. Med. Chem., 1988, 31, 645.

24. N. Acton and A. Brossi, Helv. Chim. Acta, 1980, 63, 1396.

25. P. N. Sharma and A. Brossi, ibid., 1984, 67, 301.

26. P. L. Julian and J. Pikle, J. Amer. Chem. Soc., 1935, 57, 735.

27. Q. S. Yu, J. R. Atack, S. I. Rapoport, and A. Brossi, FEBS Lett., in *press*.

28. Q. S. Yu, J. R. Atack, S. I. Rapoport, and A. Brossi, J. Med. Chem., in press.

29. Q. S. Yu and A. Brossi, Heterocycles, in press.

30. B. Schönenberger and A. Brossi, Helv. Chim. Acta, 1986, 69, 1486.

31. S. L. Wallace "Textbook on Rheumatology" (W. N. Kelly, E. D. Harris, Jr., S. Ruddy and C. B. Sledge, eds.), W. B. Saunders, Co., Philadelphia, 1983, 871-875.

32. H. G. Capraro and A. Brossi, "The Alkaloids", Vol. 23, Academic Press, New York, 1984, 1-70.

33. A. Brossi, H. J. C. Yeh, M. Chrzanowska, J. Wolff, E. Hamel, C. M. Lin, F. Quinn, M. Suffness, and J. V. Silverton, Med. Res. Reviews, 1988, 8, 77.

34. H. J. C. Yeh, M. Chrzanowska, and A. Brossi, FEBS Lett., 1988, 229, 82.

Received, 20th July, 1988