TABLE II
AMIDES, $RCONHCH(C_6H_5)_2$

R	% yield	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
H[a]	86	132–133	$C_{14}H_{13}NO$	79.60	79.75	6.20	6.06	6.63	7.03
CH_3	85	144–146[b]							
CH_3CH_2	97	140.4–141.6[c]							
CH_2=CH	70	177.8–178.8	$C_{16}H_{15}NO$	80.99	80.87	6.37	6.52	5.89	5.83
$C_6H_5CH_2$	97	161.2–162.4	$C_{21}H_{19}NO$	83.70	84.10	6.36	6.58	4.65	4.90
C_6H_5	86	171–172.4[b]							
d	64	188–192	$C_{20}H_{28}N_2O_2$	80.34	80.58	6.29	6.64	6.25	6.57
CH_2CO_2H[e]	75	94–94.4	$C_{16}H_{15}NO_3$	71.40	71.72	5.61	5.80	5.20	5.42
$CH_2CO_2C_2H_5$[f]	71	92.3–93.2	$C_{18}H_{19}NO_3$	72.71	72.58	6.44	6.39	4.71	5.12

[a] The hydrogen cyanide was prepared *in situ* from sodium cyanide. [b] H. L. Wheeler, *Am. Chem. J.*, **26**, 354 (1901); see also ref. 11. [c] W. Davies, T. H. Ramsay, and E. R. Stove, *J. Chem. Soc.*, 2633 (1949). [d] The product is N,N'-bis(benzhydryl)succinamide. [e] Starting compound, cyanoacetic acid. [f] Starting compound, ethyl cyanoacetate.

TABLE III
AMIDES, $RCONHC(C_6H_5)_3$

R	% yield	M.p., °C.
CH_3	93	206.5–207.2[a]
CH_3CH_2	91	191–192[b]
$C_6H_5CH_2$	68	187.4–188.8[c]
C_6H_5	74	159–160[d]

[a] W. Hemilian and H. Silberstein, *Ber.*, **17**, 744 (1884). [b] *Anal.* Calcd. for $C_{22}H_{21}NO$: C, 84.17; H, 6.71; N, 4.44. Found: C, 84.13; H, 6.39; N, 4.70. [c] *Anal.* Calcd. for $C_{27}H_{23}NO$: C, 85.91; H, 6.14. Found: C, 86.26; H, 6.10. [d] I. Vosburgh, *J. Am. Chem. Soc.*, **38**, 2081 (1916).

ether, was added. The reaction temperature was maintained at 50° by the use of a cooling bath; the mixture was stirred for 3 hr., allowed to stand overnight, and was then poured onto a slurry of ice–water. The solid which precipitated was filtered. In this way, 19.1 g. (85%) of product, m.p. 144–146°, was obtained.

N-Benzylacetamide.—Acetonitrile (50 ml.) and concentrated sulfuric acid (0.2 mole, 20.2 g.) were placed in the reactor; the temperature of the mixture rose to 70°. On the addition of benzyl alcohol (0.2 mole, 21.6 g.) the temperature rose to 85°. The reaction mixture was maintained at the boiling point of the acetonitrile during the addition of the alcohol. The reaction temperature was moderated with an ice bath when this was necessary. The mixture was allowed to cool to room temperature and it then was stirred for an additional 2 hr. It was poured onto a slurry of ice–water, made basic with solid sodium carbonate, and was extracted with several portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate, the solvent was removed at atmospheric pressure, and the residue was distilled *in vacuo* to give 21.7 g. (72.5%) of material, b.p. 153–156° (4.5 mm.), m.p. 60–61°.

Substrates for Cytochemical Demonstration of Enzyme Activity. I. Some Substituted 3-Indolyl-β-D-glycopyranosides[1a]

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The histochemical demonstration of nonspecific esterase in mammalian tissue through the use of substituted indoxyl esters has received considerable attention during the past decade.[2] The enzymatically released indoxyl is oxidized rapidly to an insoluble indigo which can be seen readily at the sites of activity. Recently the "indigogenic principle" was applied successfully to the histochemical localization of mammalian glucosidase through the use of 5-bromo-3-indolyl-β-D-glucopyranoside[3] for which independent syntheses had been recorded both by Anderson and Leaback[4] (method A) and the present authors[5] (method B). The methods have now been extended to some dihalogeno-3-indolyl-β-D-glycosides as part of a search for new substrates for the precise localization of mammalian glycosidases.[6]

Experimental[7]

The following procedure is considered typical of method A by which an acylohalogenoglycose may be coupled with either 1-acetyl-5-bromo-4-chloroindol-3-ol[8] or 1-acetyl-5-bromo-6-chloroindol-3-ol (*vide infra*). Deacylation of the condensation product was effected in the usual manner with catalytic quantities of sodium methoxide in an excess of dry methanol.

1-Acetyl-5-bromo-6-chloro-3-indolyl-tetra-*O*-acetyl-β-D-galactopyranoside.—A mixture of 1.81 g. (6.3 mmoles) of 1-acetyl-5-bromo-6-chloroindol-3-ol and 3.12 g. (7.6 mmoles) of tetra-*O*-acetyl-β-D-galactopyranosyl bromide in 100 ml. of acetone, cooled to 0°, was gassed for 0.5 hr. with a stream of nitrogen. A solution of 7.3 ml. of 1 *N* sodium hydroxide was added dropwise, with stirring, to the cold suspension under an atmosphere of nitrogen. The reaction mixture was stirred overnight (16 hr.) in a cold room (0°). The blue-green solution was evaporated to dryness *in vacuo* at *ca.* 30° and the oily residue solidified after extensive washing with water followed by trituration with cold ethanol. Two recrystallizations (Norit) from ethanol provided an analytical sample in the form of colorless fine needles, 1.89 g. (two crops, 49% yield), m.p. 178–179°, $[\alpha]^{23}D$ −20° (*c* 1.0, acetone).

Anal. Calcd. for $C_{24}H_{23}BrClNO_{11}$: C, 46.58; H, 4.07; N, 2.26. Found: C, 46.62; H, 4.16; N, 2.40.

5-Bromo-6-chloro-3-indolyl-β-D-galactopyranoside.—A solution of 1.0 g. (1.6 mmoles) of the acetylated product in 50 ml. of dry methanol containing 0.1 mmole of sodium methoxide was stirred overnight at 5°. The reaction mixture was neutralized with a drop of glacial acetic acid and the solution was evaporated to dryness *in vacuo* at room temperature. The residue crystallized as an amorphous, colorless powder from ethyl acetate, 0.45 g., m.p. 180–181° dec. The filtrate afforded two additional crops of material after reduction to *ca.* 0.5 of the original volume, 0.12 g. (86% total yield), m.p. 179–181° dec. A single recrystal-

(1) (a) This work was supported in part by research grants CA-02624 and CA-02903 from the National Cancer Institute, Public Health Service, and in part by an institutional grant from the United Foundation of Greater Detroit allocated through the Michigan Cancer Foundation; (b) Deceased 1962.

(2) For a review and key literature references see M. S. Burstone, "Enzyme Histochemistry and Its Application in the Study of Neoplasms," Academic Press, New York, N. Y., 1962, p. 304.

(3) B. Pearson, M. Andrews, and F. Grose, *Proc. Soc. Exptl. Biol. Med.*, **108**, 619 (1961).

(4) F. B. Anderson and D. H. Leaback, *Tetrahedron*, **12**, 236 (1961).

(5) See ref. 3.

(6) A portion of the histochemical findings has already been reported: B. Pearson, P. L. Wolf, and J. Vazquez, *Lab. Invest.*, **12**, 1249 (1963).

(7) All melting points were taken with a Thomas-Hoover apparatus and are corrected. Elementary analyses were performed by Micro-Tech Laboratories, Skokie, Ill.

(8) S. J. Holt, A. E. Kellie, D. G. O'Sullivan, and P. W. Sadler, *J. Chem. Soc.*, 1217 (1958).

lization from ether provided an analytical sample in the form of colorless needles, m.p. 180–181°, $[\alpha]^{24}D$ −41° (*c* 1.3, ethanol).

Anal. Calcd. for $C_{14}H_{15}BrClNO_6$: C, 41.15; H, 3.70; N, 3.43. Found: C, 41.00; H, 3.89; N, 3.45.

5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside crystallizes as an amorphous solid from methanol, m.p. 237–239° dec., $[\alpha]^{24}D$ −69° (*c* 1.0, 50% DMF).

Anal. Found: C, 41.24; H, 3.78; N, 3.63.

5-Bromo-6-chloro-3-indolyl-β-D-glucopyranoside crystallizes as a mat of colorless needles from methanol, m.p. 196–198°, $[\alpha]^{24}D$ −60° (*c* 1.0, acetone).

Anal. Found: C, 40.81; H, 3.86; N, 3.47.

5-Bromo-4-chloro-3-indolyl-2-deoxy-D-*arabino*-hexapyranoside[9] crystallizes from ethanol as colorless needles, m.p. 211–212°, $[\alpha]^{26}D$ −106° (*c* 1.0, ethanol).

Anal. Calcd. for $C_{14}H_{15}BrClNO_5$: C, 42.83; H, 3.85; N, 3.57. Found: C, 42.54; H, 4.01; N, 3.56.

5-Bromo-4-chloro-3-indolyl-β-D-glucopyranoside (Method B).—To 65 ml. of cold (−5°) dry methanol containing 0.6 g. (0.026 g.-atom) of sodium and under an atmosphere of nitrogen was added, all at once, 7.75 g. (0.026 mole) of 5-bromo-4-chloro-3-indolyl acetate[10] and the stirred mixture was gassed for 0.5 hr. with a stream of dry nitrogen. A solution of 11 g. (0.027 mole) of acetobromoglucose in 65 ml. of methanol was then added dropwise at a rapid rate to the stirred solution while maintaining an external temperature of 0°. After 18 hr., during which time the reaction mixture was allowed to reach room temperature, the solvent was evaporated *in vacuo* at *ca.* 30°. The viscous yellow oil that remained solidified slowly on stirring with water and the dark product was collected and sucked dry. The filter cake was stirred with cold acetone which removed most of the dark material, leaving an off-white solid, 2.6 g. (25% yield), m.p. 240–243° dec. Two recrystallizations from methanol provided an analytical sample, m.p. 240–243° dec., $[\alpha]^{23}D$ −89° (*c* 1.0, 50% DMF).

Anal. Calcd. for $C_{14}H_{15}BrClNO_6$: C, 41.15; H, 3.70; N, 3.43. Found: C, 41.16; H, 3.89; N, 3.39.

1-Acetyl-5-bromo-6-chloroindol-3-ol.—To 45 ml. of 90% sulfuric acid was added, portionwise, with continuous stirring, 9.5 g. (0.029 mole) of 1-acetyl-5-bromo-6-chloro-3-indolyl acetate[10] while maintaining an internal temperature of 20–25°. After 0.75 hr., the reaction mixture was poured on ice, and the yellow solid was collected and washed with generous quantities of a 1% solution of sodium acetate. The vacuum-dried (P_2O_5) product, 7.6 g. (91% yield), m.p. 218–221° dec., afforded analytical material, in the form of pale yellow plates, after first crystallizing from a large volume of ethyl acetate followed by two recrystallizations from ethanol, m.p. 238–239° dec., λ_{max}^{Nujol} 5.82 (amide carbonyl) and 6.0 μ [3-(enolic)carbonyl].

Anal. Calcd. for $C_{10}H_7BrClNO_2$: C, 41.62; H, 2.45; N, 4.85. Found: C, 41.44; H, 2.52; N, 4.97.

(9) The bromo sugar component in this case was the stable 3,4,6-tetra-*O*-(*p*-nitrobenzoyl)-α-D-*arabino*-hexopyranosyl bromide which was recently reported by W. W. Zorbach and G. Pietsch [*Ann.*, **655**, 26 (1962)]. It is assumed, though it is in no way certain, that the product is the β-anomer.

(10) S. J. Holt and P. W. Sadler, *Proc. Roy. Soc.* (London), **B148**, 492 (1958); S. J. Holt in "General Cytochemical Methods," J. F. Danielli, Ed., Academic Press, New York, N. Y., 1958, p. 375.

Book Reviews

Handbuch der Experimentellen Pharmakologie. Volume 15. **Cholinesterases and Anticholinesterase Agents.** Sub-Editor, GEORGE B. KOELLE. Contributors: K. B. Augustinsson, L. E. Chadwick, J. A. Cohen, H. Cullumbine, D. R. Davies, K. P. DuBois, D. Grob, C. O. Hebb, F. Hobbiger, Bo Holmstedt, A. G. Karczmar, G. B. Koelle, N. Krishna, A. S. Kuperman, I. H. Leopold, J. P. Long, X. Machne, L. A. Mounter, D. Nachmansohn, R. A. Oosterbaan, K. R. W. Unna, G. Werner, V. P. Whittaker, J. H. Wills, and E. Zaimis. 1220 pp. Springer-Verlag, Berlin–Gottingen–Heidelberg. 1963. $74.50.

Since the publication of the first paper on cholinesterases (CHE) in 1914, considerable progress has been made on the specificity and the physiological role of these enzymes and their inhibitors, especially in the last 30 years. It is pointed out by one of the contributors that about 200–300 papers have been published each year since 1950 on CHE and antiCHE compounds. The rapid progress made, and the voluminous information published in this area make it very difficult for an individual scientist to keep up with all the developments. Perspective in such a vast subject is gained best by broad reviews written by experts or teams of experts on various aspects related to the area. The fifteenth volume in the series "Handbuch der Experimentellen Pharmakologie" offers an admirable attempt to cover the broad area of CHE and antiCHE compounds.

This volume is divided into four major sections. Each section is subdivided into four to seven chapters, and the whole text contains 24 chapters. Most of the chapters are very comprehensive, and within their chosen or allocated chapters, the authors had clear scope to develop their themes as seemed best to them. The book has adequate author and subject indexes.

Section I.—Components of cholinergic systems: acetylcholine (ACH), choline acetylase (CHAC), and acetylcholinesterase (ACHE). This section contains six chapters. The chapter by Whittaker is an extensive discussion on the analytical methods for the identification, detection, and estimation of cholinesters. The biochemical aspects of CHAC is discussed by Nachmansohn. The formation, storage and liberation of ACH is presented by Hebb. Augustinsson's chapter is a comprehensive review of the classification and the comparative enzymology of the types of CHE and the methods for their determination. The significance of ontogenetic appearance of CHE, and relationships between CHE, neurogenesis, and function is discussed by Karczmar. The chapter by Koelle includes much detail on the methods available for the cytological localization of ACHE and non-specific CHE. This chapter also contains an excellent discussion of the physiological functions of ACH and ACHE.

Section II.—Chemical classification and biochemical reactions of the anticholinesterase agents. This section contains four chapters. Cohen and Oosterbaan have given a lucid account of the interaction of ACHE with substrates and inhibitors, and the chemical analysis of the active sites of esterases related to ACHE. Long has provided an enormous amount of information on the relationships between the chemical structure and the reversible antiCHE properties of many classes of chemical compounds, making use of classification and tabular presentation. This author has clearly pointed out the requirements for making valid structure-activity relationship (SAR) comparisons and the limitations of such studies. Holmstedt's chapter is devoted to the SAR of the organophosphorus antiCHE agents. This chapter includes, unexpectedly but appropriately, a historical account of the development of the organophosphorus inhibitors in industries, universities, and military research institutes. The various chemical reactions in the metabolism of organophosphorus antiCHE agents by mammals and microorganisms are discussed by Mounter.

Section III.—Systematic pharmacology of antiCHE agents. This section contains seven chapters. The actions of antiCHE compounds on the secretory glands, smooth muscle, and the cardiovascular system are summarized by Cullumbine. The chapter by Zaimis deals with the actions of antiCHE compounds at autonomic ganglia and the theories regarding the release of ACH at ganglia. Werner and Kuperman have reviewed the actions of antiCHE compounds at the neuromuscular junction of amphibian, avian, and mammalian muscle. In their discussion, these authors have included information on the nature of the types of CHE at the neuromuscular junction, potentiation of contractile response by antiCHE compounds, and the antidromic activity in the motor nerves. The chapter by Machne and Unna is devoted to the actions of antiCHE compounds in certain functional areas of the brain, including midbrain reticular formation and spinal cord. Nachmansohn has discussed the evidence for the role of ACH in axonal conduction of nerve impulse. The actions of antiCHE compounds on insects and other invertebrates is summarized by Chadwick. Karczmar has reviewed the morphogenetic, toxic, and the melanophore effects of antiCHE agents during ontogenesis. He has included in his discussion the actions of antiCHE agents on function during development.

Section IV.—Toxicology and therapeutic applications of the anticholinesterases. This section contains seven chapters. The