Organic Sulfate Esters of Potential Biological Interest

Maxwell Gordon and Ann S. Hunter

Smith Kline and French Laboratories

Dedicated to Professor Allan R. Day

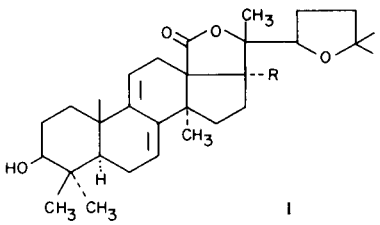
The area of organic sulfate esters of potential biological interest has been the subject of a number of reviews (19, 35,40,47). We would like to highlight the recent literature of this evolving field.

Most of the organic sulfates of this review are glycosides or polysaccharides and the sulfate ester is commonly attached to a sugar unit, although in the case of steroid metabolites it may be attached to a steroid alcohol. The glycoside or polysaccharide sulfates occur widely in the plant and animal kingdoms. Inasmuch as the sulfate-free analogs are often biologically less active than the sulfate esters, their biological activity may be more related to their physical properties than to their chemical reactivity.

Although carbohydrate sulfates occur frequently in marine algae and in marine plants, they are rarely found in higher plants. The "plant sulfolipid" seen in higher plants is actually a sulfonic acid, not a sulfate ester (35).

Steroidal Saponins.

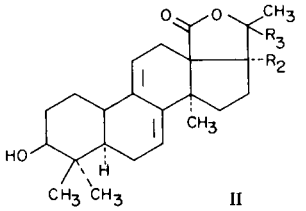
The pioneering work of Friess and his group (13,14,15) on Holothurin has aroused great interest in this family of substances. Many species of Holothuroidea, in the family of the sea cucumber contain glycoside sulfates which are highly potent neurotoxins, whose activity in blocking elicited twitch response of muscle, is dependent on the presence of the sulfate group (13,14). Holothurin, the class name of a group of toxic substances of *Actinopyga agassizi*, is a mixture containing glycoside sulfates. These yield, on hydrolysis, xylose, quinovose, glucose, 3-*O*-methylglucose, sulfuric acid, and a mixture of aglycones including I and II (4,5,26,38,39,50).



I

Ia R = OH 22,25-oxidoholothurinogenin

Ib R = H 17-desoxy-22,25-oxidoholothurinogenin



II

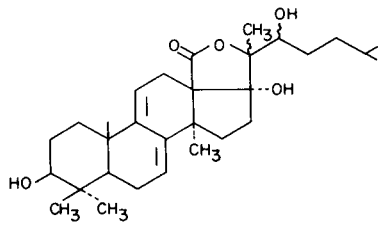
R_2 = H, OH

R_3 = (a) $CHOHCH_2\ CH_2\ CH(CH_3)_2$

(b) $CH_2\ CH_2\ CH{=}C(CH_3)_2$

(c) $CH{=}CHCH_2\ CH(CH_3)_2$

A related sapogenin, griseogenin (III) from the sea cucumber, *Halodeima grisea*, has been isolated by Tursch, *et.al.*, (46). This may be identical with IIa.



III

Asterosaponin A, a steroidal saponin from the starfish, *Asterias amurensis*, has been studied with respect to potency in producing irreversible blockade of contractural responses from a rat phrenic nerve-diaphragm preparation (15). Asterosaponin A is relatively more potent in blockade of indirectly elicited contraction (N-twitches) than against responses provoked by direct muscle stimulation (M-twitches). The second saponin, Asterosaponin B, showed an inverted effect with greater activity in blocking muscular twitches than indirectly elicited contractions, M-twitch >N-twitches. Friess, *et.al.*, (15) have interpreted these observations in terms of surface polarity differences among the sugars comprising model glycosidic chains in the two structures.

It is of interest that a variety of marine organisms synthesize steroidal saponins and employ them for offensive or defensive purposes. Thus as mentioned above *Actinopyga agassizi* contains two principles, Holothurin A and

TABLE I

Structural Properties of Purified Echinoderm Saponins

Saponin	Animal Source	Reference	Presence of Sulphate group	Sugars per molecule	Approx Molecular Weight
Holothurin A	Actinopyga agassizi Selenka	(4,5,6,7)	Yes	1 D-Xylose 1 D-Glucose	1159
	H. vagabunda	(51)	Yes	1 3-*O*-Methyl-D-Glucose	1279
	H. lubrica			1 D-Quinovose	
Holothurin B	H. vagabunda	(51)	Yes	1 D-Xylose	991
	H. lubrica	(51)		1 D-Quinovose	
Asterosaponin A	Asterias Amurensis Lütken	(50)	Yes	2 D-Quinovose 2 D-Fucose	1141
Asterosaponin B	Asterias amurensis Lütken	(52)	Yes	2 D-Quinovose 1 D-Fucose 1 D-Xylose 1 D-Galactose	1341

Holothurin B, which effect irreversible blockade of cholinergic neuromuscular transmission, and also evoke direct contractural responses from striated muscle.

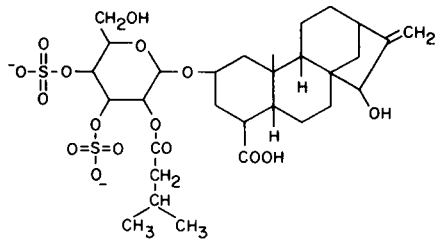
The Table I taken from Friess, *et.al.*, (15) summarizes the chemical data on these sulfate esters.

A comparison of Holothurin A with its sulfate-free analog (DeH) is of interest. Thus DeH is able to afford significant elements of protection against the irreversible destruction of the twitch response normally evoked by Holothurin A at a 10^{-4}M level. However, DeH did not protect mice against the lethality of Holothurin A.

Asterosaponin A and B have undergone structural investigations by Yasumoto and Hashimoto (50,51,52). The first contains the sugars D-quinovose and D-fucose, together with one sulfate residue for every four sugars, plus a conjugated ketone. Asterosaponin B contains a conjugated ketone, one molecule of sulfuric acid, two molecules of D-quinovose and one molecule each of D-fucose, D-xylose, and D-galactose. The asterosaponins may be structurally related to Holothurin.

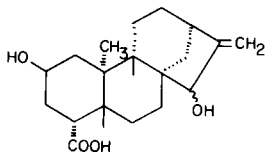
Atractyloside (IV).

Atractyloside is a toxic glycoside sulfate (32,33,34) isolated from *Atractylis gummifera*, which inhibits, at very low doses, respiration when coupled to phosphorylation. The sulfate-free aglycone, atractyligenin, is less active biologically.



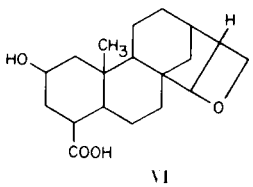
IV

The structure and stereochemistry of atractyligenin was elucidated by a number of investigators (16,17,21,32, 32,33,34) and confirmed by partial synthesis. Stereochemical assignments for atractyligenin are shown in compound V. This compound can be isomerized with zinc and base



V

to give isoatractyligenin (VI).

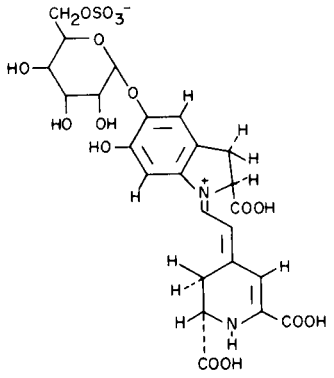


VI

The skeleton of atractyligenin has been identified with the diterpene kaurane (16,17).

Prebetanin.

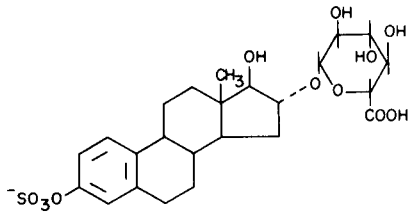
Prebetanin (VII), a sulfate half ester of betanin, has been isolated as a minor component of the red beet (49). Little is known about biological activity in this series:



VII

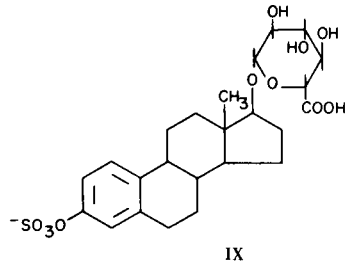
Steroid Conjugate Sulfates.

Joseph, *et.al.*, (23) have synthesized a sulfoglucuronide derivative of estriol (VIII), which, with related compounds, may play a role in estrogen endocrinology and metabolism. Previously Cantrall, *et.al.*, (2)



VIII

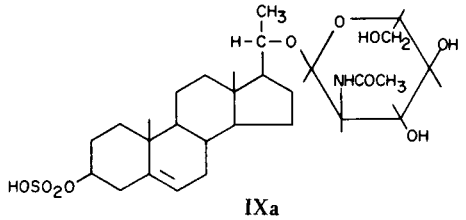
synthesized an estradiol sulfate glucuronide (IX).



IX

Other sulfates of estrogen derivatives have been prepared by Miyazaki and Fishman (28).

Arcos and Liebermann (1) have isolated two crystalline steroid conjugates from the urine of normal human subjects to whom pregnenolone was administered. One of these metabolites was 5-pregnene-3β,20α-diol-3-sulfate-20-(2′-acetamido-2′-deoxy-α-D-glucoside) (IXa).



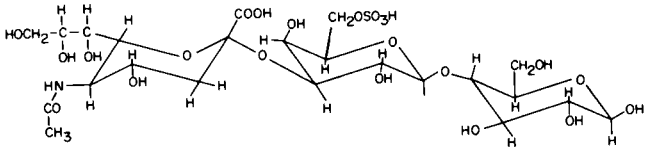
IXa

This is apparently the first time that a steroid metabolite conjugated with D-*N*-acetylglucosamine has been isolated from human urine. The second new metabolite of pregnenolone was the disulfate of pregnenediol. Of the pregnenolone produced *in vivo*, 8% is excreted as the above two conjugates.

Although adenosine-3′-phosphate-5′-phosphosulfate has been established as the source of "active" sulfate in the enzymic conjugation of steroids, Chu and Slaunwhite (8) were interested in the decrease in ascorbic acid during adrenal steroidogenesis and synthesized ascorbic acid-3-sulfate to study its role as a chemical sulfating agent. They found that *in vitro* reaction of this sulfating agent with androsterone produced androsterone sulfate.

Neuraminyl Lactose Sulfate.

This constituent of aqueous extracts of lactating rat mammary glands has been shown to have structure X (36).



X

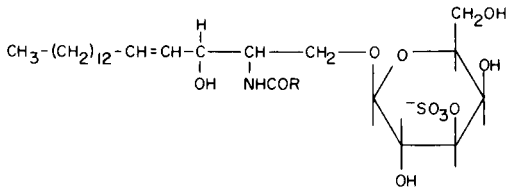
Inasmuch as neuraminic acid is an important constituent of glycoproteins and other mammalian constituents, its sulfate esters may be presumed to play a biological role.

Additional sulfates that have been isolated from rat mammary gland extracts include lactose sulfate (*O*-β-D-galactopyranosyl-6-*O*-sulfate-(1→4) D-glucopyranose), identical in structure with the lactose sulfate portion of neuraminyl lactose sulfate.

Sulfatides.

Sulfate esters of cerebrosides are known as sulfolipids or sulfatides, and they are found in the brain and other tissues (3). In addition, a disaccharide cerebroside sulfate has been isolated from human kidney (25). Flowers (12)

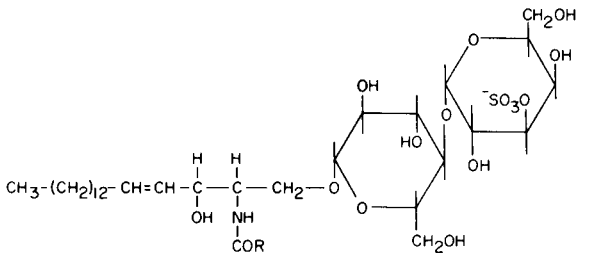
has synthesized a dihydrosulfatide from the dihydrocerebroside derivative, 1-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-3-*O*-benzoyl-*N*-octadecanoyl-*dl*-dihydrosphingosine. A sulfatide structure is shown as XI.



XI

It has been suggested that cerebroside sulfates are formed by enzymatic sulfate transfer from 3′-phosphoadenosine, 5′-phosphosulfate to cerebroside (35). A deficiency of sulfatase is thought to lead to a metabolic disorder, metochromatic leukodystrophy, involving an abnormal accumulation of sulfatides in the CNS (40). Suzuki, *et.al.*. (42) reported on the isolation of metachromatic granules from a brain with metachromatic leukodystrophy. Lipids in these granules consisted of a 1:1:1 molar ratio of cholesterol, galactolipids and phosphatides. About 85% of the galactolipids were sulfatides. Gangliosides did not appear to be constituents of the granule membranes.

Stoffyn, *et.al.*, (41) have established that kidney ceramide dihexoside sulfate is a lactosyl ceramide with the sulfate group at C-3 of the galactose moiety (XIa).



XIa

This sulfatide, containing equal parts of ceramide, glucose, galactose, and sulfate, constitutes 25-30% of the total sulfatide fraction of human kidney. This ceramide dihexoside sulfate is also present in increased amounts in patients with metachromatic leukopystrophy, but it accumulates in kidney at a slower rate than the more abundant ceramide galactose sulfate. The biological role played by these sulfatides in health and disease states is not known.

Two general types of cerebral sulfatides which are known are shown below (22):

Cerebron type	R=	$CH(OH)\text{-}(CH_2)_{21}\text{-}CH_3$
	R=	$CH(OH)\text{-}(CH_2)_{12}\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}CH_3$
Kerasin type	R=	$(CH_2)_{22}CH_3$
	R=	$(CH_2)_{13}\text{-}CH{=}CH\text{-}(CH_2)_7CH_3$

Changes have been reported in the fatty acid composition of cerebrosides and sulfatides of human nervous tissue with age (9,26,45).

Thus McKann and Ho (26) showed that S^{35} sulfate injected i.p. into young rats was taken up into the lipid fraction of whole brains of 15-20 day old rats at a maximum rate, corresponding to the rate of maximum myelin synthesis. The developmental pattern of the galactocerebroside sulfotransferase of the kidney was different from that of the brain, development being closely associated with kidney weight. Cumar, *et.al.*, also demonstrated that preparations from the brain of young rats contain enzymes that catalyze the transfer of sulfate S^{35} from 3′-phosphoadenosine-5′-phosphosulfate-S^{35} to galactose-containing sphingolipids, as well as other enzymes that catalyze sulfate transfer to galactose and water soluble galactosides.

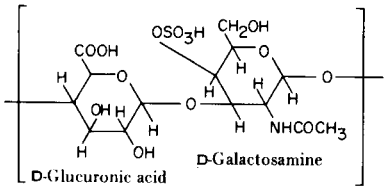
Svennerholm, *et.al.*, (45) studied isolation of sulfatides from human brain at various ages and found that infant brain galactolipids had a lower percentage of $C_{22}\text{-}C_{26}$ fatty acids and monoenoic acid than that of adults or children. Low activities of fatty acid elongation and desaturation systems during myelination were inferred. Fatty acid changes with age were the same for cerebrosides and sulfatides, but occurred later in the latter, indicating that cerebrosides are precursors of sulfatides.

Kates, *et.al.*, (24) have isolated a new glycolipid sulfate ester from *Halobacterium cutirubrum*, containing 3 moles of sugar per sulfate residue, and a 2,3-di-*O*-phytanyl-L-glycerol moiety. Further structure work is underway.

Polysaccharide Sulfates.

A number of carbohydrate sulfates of mammalian origin have been identified including chondroitin-4-sulfate (XII) (XIII) and chondroitin-6-sulfate (XIV) from hyaline cartilage, dermatan sulfate (XIII) from skin, heart and lungs, keratan sulfate (XV) from liver (43) heparin sulfate (XVI) from liver and lungs, and other unidentified carbohydrate sulfates in mammalian brain, gastric juice, gastric wall, etc. (35).

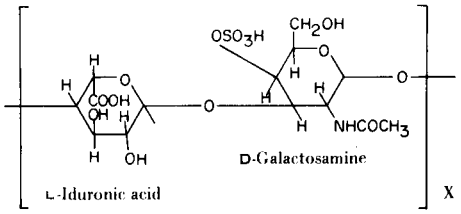
Chondroitin sulfate-A (4-sulfate) (XII)



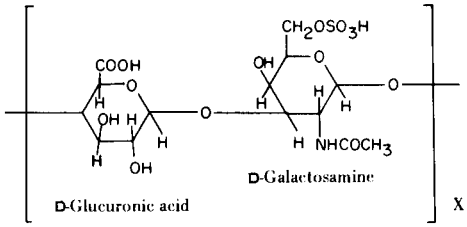
D-Glucuronic acid D-Galactosamine

X

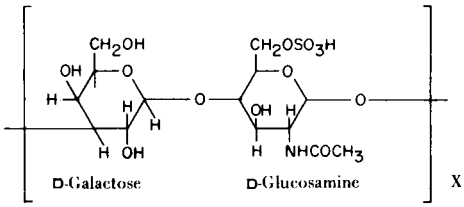
Chondroitin sulfate-B (4-sulfate) (XIII) (Dermatan sulfate) (β-Heparin)



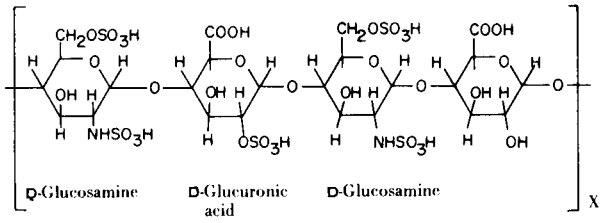
Chondroitin sulfate-C (6-sulfate) (XIV)



Keratan sulfate (XV)



Heparin Sulfate (XVI)



Analysis of human brain of different age groups showed that levels of chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, and two unidentified low sulfated fractions rose from fetal levels to a peak at one year of age, then fell. Thus these compounds also play a roll in myelination and brain maturation (37).

Turvey (47) has listed a number of sulfates of simple sugars that occur in nature or have been synthesized, including the 2-,3-,4- and 6-sulfates of D-glucose, as well as their methylpyranosides or furanosides, 2,3,4, and 6-sulfates of galactose and D-galactose-2,3-disulfate (30).

Suzuki, *et.al.*, (43,44) have isolated three types of disulfated disaccharides from chondroitin sulfates by chondroitinase digestion.

Miscellaneous.

Polysaccharide sulfates have been isolated from blue-green algae. These include L-arabinose-D-galactose sulfates, D-glucuronic acid L-rhamnose-D-xylose sulfates, etc. (29).

Cyclic sulfates in the sugars and cerebrosides (10,20) have been suggested and it is interesting to speculate on the biological role of these compounds, in view of the importance of cyclic adenosine monophosphate.

Some publications on the synthesis of sugar sulfates include Whistler, *et.al.*, (48); Fex, *et.al.*, (11); and Gilbert (18).

Recently Ondetti (53) reported on the synthesis of cholecystokinin-pancreozymin, confirming the proposed structure from degradative experiments. The eighth amino acid in this decapeptide is a tyrosine bearing a sulfate ester on the phenolic hydroxyl group. If the sulfate residue is removed, most of the biological activity is lost. Similarly, an octapeptide fragment, terminating in the sulfated tyrosine, which has about half the activity of the native peptide, loses most of its biological activity upon removal of the sulfate group.

SUMMARY

This review mentions a variety of naturally occurring sulfates, which seem to be worthy of further study from the viewpoint of their biological activity. One class of compounds comprises physiologically highly-active (or toxic) steroidal saponins produced by lower animals or plants, ostensibly for protective purposes. In these cases the organic sulfates are much more active than their sulfate-free analogs.

A second class of sulfates includes substances whose organic sulfates serve as depot forms or in a detoxication (solubilization) mechanism. Compounds of this class include natural sterols (estrogens and androgens), tyrosine, indoxyl, and amino sugars, in addition to a host of foreign substances like phenols, synthetic drugs, etc.

A final group of substances includes membrane and structural carbohydrate sulfates like sulfatides, chondroitins, heparin, etc., where the role of the sulfate group is essential, but obscure. Of particular recent interest is a pancreatic enzyme (cholecystokinin-pancreozymin) which requires a sulfate ester on a tyrosine moiety for biological activity.

REFERENCES

(1) M. Arcos and S. Lieberman, *Biochemistry*, 6, 2032 (1967).

(2) E. W. Cantrall, M. G. McGrath and S. Bernstein, *Steroids*, 8, 967 (1966).

(3) H. E. Carter, P. Johnson and E. J. Weber, *Ann. Rev. Biochem.*, 34, 109 (1965).

(4) J. D. Chanley, *J. Am. Chem. Soc.*, 81, 5180 (1959).

(5) J. D. Chanley and H. Sobotka, *Intern. Symp. Org. Chem.*

Natural Prod., Ind. Chem. Belg., 532, (1962).
(6) J. D. Chanley, T. Mezzetti and H. Sobotka, *Tetrahedron*, 22, 1857 (1966).
(7) H. U. Choi and R. Carubelli, *Biochemistry*, 7, 4423 (1968).
(8) T. M. Chu and W. R. Slaunwhite, *Steroids*, 12, 309 (1968).
(9) F. A. Cumar, H. S. Barra, H. J. Maccione and R. Caputto *J. Biol. Chem.*, 243, 3807 (1968).
(10) A. N. Davison, *Biochem. J.*, 91, 3p (1964).
(11) H. Fex, K. E. Lundvall and A. Olsson, *Acta Chem. Scand.*, 22, 254 (1968).
(12) H. M. Flowers, *Carbohydrate Res.*, 2, 371 (1965).
(13) S. L. Friess and R. C. Durant, *Toxicol. Appl. Pharmacol.*, 7, 373 (1965).
(14) S. L. Friess, R. C. Durant, J. D. Chanley and F. J. Fash, *Biochem. Pharmacol.*, 16, 1617 (1967).
(15) S. L. Friess, R. C. Durant and J. D. Chanley, *Toxicon.*, 6, 81, Pergamon Press, London (1968).
(16) E. L. Ghisalberti and P. R. Jeffries, *Austr. J. Chem.*, 21, 439 (1968).
(17) E. L. Ghisalberti, P. R. Jeffries, S. Passanati, and F. Piozzi, *ibid.*, 21, 459 (1968).
(18) E. E. Gilbert, *Chem. Rev.*, 62, 549 (1962).
(19) I. H. Goldberg, *J. Lipid Res.*, 2, 103 (1961).
(20) S. Hakomori, T. Ishimoda and K. Nakamura, *J. Biochem.* (Tokyo), 52, 468 (1962).
(21) J. R. Hanson and A. F. White, *Tetrahedron*, 24, 2533 (1968).
(22) H. Jatzkewitz and G. Nowocyzek, *Chem. Ber.*, 100, 1667 (1967).
(23) J. P. Joseph, J. P. Dusza and S. Bernstein, *J. Am. Chem. Soc.*, 89, 5078 (1967).
(24) M. Kates, B. Palameta, M. P. Perry and G. A. Adams, *Biochem. Biophys. Acta*, 137, 213 (1967).
(25) E. Martensson, *ibid.*, 116, 521 (1966).
(26) T. Matsuno and J. Iba, *Yakugaku Zasshi.*, 86, 637 (1966).
(27) G. M. McKann and W. Ho, *J. Neurochem.*, 14, 717 (1967).
(28) M. Miyazaki and J. Fishman, *J. Org. Chem.*, 33, 662 (1968).
(29) S. Peat and J. R. Turvey, *Fortschr. Chem. Organ. Naturst.*, 23, 30 (1965).
(30) S. Peat, D. M. Bowker and J. R. Turvey, *Carbohydrate Res.*, 7, 225 (1968).
(31) R. Piozzi, *et.al.*, *Tetrahedron Letters*, 1829 (1965).
(32) F. Piozzi, A. Quilico, R. Mondelli, T. Ajello, V. Sprio and A. Melera, *Tetrahedron Suppl.* 8, Part II, 515 (1966).
(33) F. Piozzi, A. Quilico, R. Mondelli, T. Ajello, V. Sprio and A. Melera, *Chim. Ind.* (Milan), 48, 371 (1966).
(34) F. Piozzi, A. Quilico, C. Fuganti, T. Ajello and V. Sprio, *Gazz. Chim. Ital.*, 97, 935 (1967).
(35) D. A. Rees, *Ann. Repts. Progr. Chem.*, 62, 469 (1965).
(36) L. C. Ryan, R. Carubelli, R. Caputto and R. E. Trucco, *Biochem. Biophys. Acta.*, 101, 252 (1965).
(37) M. Singh and B. K. Bachhawat, *J. Neurochem.*, 15, 249 (1968).
(38) H. Sobotka, *Chem. and Chem. Industry* (Japan), 16, 1023 (1963).
(39) H. Sobotka, *Bull. Soc. Chim. Biol.*, 47, 169 (1965).
(40) P. J. Stoffyn, *J. Am. Oil Chem.*, 43, 69 (1966).
(41) A. Stoffyn, P. Stoffyn and E. Martensson, *Biochem. Biophys. Acta*, 152, 353 (1968).
(42) K. Suzuki, K. Suzuki and G. C. Chen, *J. Neuropathol. Exp. Neurol.*, 26, 537 (1967).
(43) K. Suzuki, *Science*, 159, 1471 (1968).
(44) K. Suzuki, H. Saito, T. Yamagata, K. Anno, N. Seno, Y. Kawai and T. Furuhashi, *J. Biol. Chem.*, 243, 1543 (1968).
(45) L. Svennerholm and S. Stallberg-Stenhagen, *J. Lipid Res.*, 9, 215 (1968).
(46) B. Tursch, *Tetrahedron*, 23, 761 (1967).
(47) J. R. Turvey, *Adv. in Carbohydrate Chem.*, 20, 183 (1965).
(48) R. L. Whistler, A. H. King, G. Ruffini and F. A. Lucas, *Arch. Biochem. Biophys.*, 121, 358 (1967).
(49) H. Wyler, H. Rösler, M. Mercier and A. S. Dreiding, *Helv. Chim, Acta.*, 50, 545 (1967).
(50) T. Yasumoto and Y. Hashimoto, *Agr. Biol. Chem.*, 31, 368 (1967).
(51) T. Yasumoto, K. Nakamura and Y. Hashimoto, *ibid.*, 31, 7 (1967).
(52) T. Yasumoto and Y. Hashimoto, *ibid.*, 29, 804 (1965).
(53) M. A. Ondetti, 158th Meeting American Chemical Society, New York, Sept. 8, 1969, Symposium on agents affecting gastrointestinal function.

Note added in proof:

The following recent references on Holothurin have appeared:

J. D. Chanley and C. Rossi, *Tetrahedron*, 25, 1897 (1969), The Holothurinogenins - II, Methoxylated Neo-holothurinogenins.

J. D. Chanley and C. Rossi, *ibid.*, 25, 1911 (1969), The Neoholothurinogenins - III, Neo-holothurinogenins by Enzymatic Hydrolysis of Desulfated Holothurin A.

The following additional references on sulfates have appeared:

G. L. Mayers, M. Pousada and T. H. Haines, *Biochemistry*, 8, 2981 (1969), Microbial Sulfolipids. III. The Disulfate of 1,14-Docosanediol in *Ochromonas danica*.

D. G. Young and P. F. Hall, *ibid.*, 8, 2987 (1969), The Side-Chain Cleavage of Cholesterol and Cholesterol Sulfate by Enzymes from Bovine Adrenocortical Mitochondria.

S. Bernstein, R. B. Conrow, J. P. Dusza and J. P. Joseph, 158th Meeting of the American Chemical Society, N. Y., Sept. 1969, Chemical Aspects of Steroid Conjugation (sulfates).

S. Lieberman, 158th Meeting of the American Chemical Society, N. Y., Sept. 1969, The Metabolic Significance of the Sulfates of 3-Hydroxysteroids.

Received July 22, 1969 Philadelphia, Pa. 19101