Cholecystokinin Antagonists. Synthesis of Asperlicin Analogues with Improved Potency and Water Solubility

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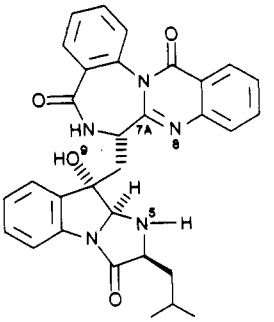
Seventeen analogues of the selective, competitive cholecystokinin (CCK) antagonist asperlicin 1 were prepared. These compounds were tested as inhibitors of the binding of [^{125}I]CCK to rat pancreas and guinea pig brain receptors. Compounds 4, 7, and 8 were more potent than asperlicin on the pancreatic CCK receptor. One analogue, 17, displayed potency equivalent to asperlicin on the pancreas CCK receptor and showed a marked improvement in aqueous solubility, thereby facilitating the use of this class of CCK antagonists in physiological and pharmacological studies.

Cholecystokinin (CCK) is a 33 amino acid neuropeptide[1] that exists in gastrointestinal tissue and in the central nervous system.[2] This agent is a major hormonal stimulator of gall bladder contraction and pancreatic exocrine secretion and has been recognized as a regulator of gut motility.[3] Additionally, CCK is known to produce satiety and, therefore, may be a key factor in the physiological regulation of appetite.[4] CCK has also been reported to coexist with dopamine in certain midbrain neurons and thus may play an equally important role in the functioning of dopaminergic systems in the brain, as well as serving as a neurotransmitter.[5]

The discovery of asperlicin 1, a new selective, competitive nonpeptide antagonist of CCK has recently been reported.[6] The demonstrated high in vitro and in vivo potency and selectivity of asperlicin for peripheral CCK receptors confer clear advantages over previously reported CCK antagonists as a tool for investigating the physiological and pharmacological actions of CCK. However, the use of asperlicin for such purposes can be limited by its very low solubility in aqueous media. The inherent nonspecific activities and toxicity of most organic solvents can be a factor that determines the magnitude of the doses of asperlicin that can be studied and the types of experiments that can be performed (e.g., iontophoresis). In this report, we disclose the results of the first analogue study on this new chemical class of CCK antagonist represented by asperlicin (1). The selected chemical transformations carried out on 1 were designed to assess which structural features of asperlicin could be modified to further enhance its CCK inhibitory potency without compromising its peripheral selectivity. It was an additional aim in this work to prepare a derivative of 1 that displayed sufficient water solubility to facilitate its in vivo and in vitro utility as a pharmacological tool.

Results and Discussion

Chemistry. At the outset of our analogue study on asperlicin (1) we sought to gauge its chemical reactivity under a variety of conditions. An analysis of the structure of 1 revealed four sites likely to be amenable to selective chemical derivatization and/or functional group interchange. These sites are the 7A–8 imine bond in the quinazoline portion of the molecule, the secondary amide linkage in the seven-membered ring, the tertiary hydroxyl group, and the secondary amine of the leucine annulated to the indole nucleus. The initial pilot reactions, each designed to involve one or more of these primary sites, gave results that were informative and in some cases unexpected. For example, 1 showed surprising stability toward



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acids and was recovered unchanged after exposure to trifluoroacetic acid (23 °C, 3 days) and hydrochloric acid (1 N, 105 °C, 12 h). Attempts to deliberately dehydrate 1 under a variety of other conditions met with mixed results. Treatment of 1 with the Burgess reagent[7] afforded uncharacterizeable polar products whereas reaction with thionyl chloride in pyridine (0–23 °C) gave the unstable olefin 2. Asperlicin was also exposed to a number of reducing conditions. Hydrogenation with 10% palladium catalyst (40–45 psi, 23 °C) in either ethanol or acetic acid left 1 unchanged; however, treatment of 1 with lithium aluminum hydride or diborane in tetrahydrofuran yielded multiple component mixtures (>10 products). Reaction of 1 with stoichiometric amounts of alkyllithium and Grignard reagents gave analogous results; in each instance, complex reaction mixtures were obtained. Finally, alkylation of 1 with dimethyl sulfate gave both N-methylated

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(1) (a) Mutt, V.; Jorpes, J. E. *J. Biochem.* **1971**, *125*, 678. (b) Mutt, V.; Jorpes, J. E. *Eur. J. Biochem.* **1968**, *6*, 156.
(2) (a) Rehfeld, J. F. *J. Neurochem.* **1985**, *44*, 1. (b) Jorpes, J. E.; Mutt, V. In *Secretion, Cholecystokinin, Pancreozymin, and Gastrin*; Jorpes, J. E., Mutt, V., Eds.; Springer-Verlag: New York, 1973; p 1. (c) Mutt, V. In *Gastrointestinal Hormones*; Glass, G. B. J., Ed.; Raven: New York, 1980; p 169.
(3) Williams, J. A. *Biomed. Res.* **1982**, *3*, 107.
(4) Smith, G. P. In *Eating and Its Disorders*; Stunkard, A. J., Stellar, E., Eds.; Raven: New York, 1984; p 67.
(5) (a) Prange, A. J.; Nemeroff, C. B. *Annu. Rep. Med. Chem.* **1982**, *17*, 31 and references cited therein. (b) Morley, J. E. *Life Sci.* **1982**, *30*, 479.
(6) (a) Albers-Schönberg, G.; Chang, R. S. L.; Lotti, V. J.; Chen, T. B.; Monaghan, R. L.; Birnbaum, J.; Stapley, E. O.; Goetz, M. A.; Lopez, M.; Patchett, A. A.; Liesch, J. M.; Hensens, O. D.; Springer, J. P. Proceedings of the Ninth American Peptide Symposium, Toronto, Canada, June 23–28, 1985. (b) Chang, R. S. L.; Lotti, V. J.; Monaghan, R. L.; Birnbaum, J.; Stapley, E. O.; Goetz, M. A.; Albers-Schönberg, G.; Patchett, A. A.; Liesch, J. M.; Hensens, O. D.; Springer, J. P. *Science (Washington, D.C.)* **1985**, *230*, 177.
(7) Burgess, E. M.; Penton, H. R., Jr.; Taylor, E. A. *J. Org. Chem.* **1973**, *38*, 26.

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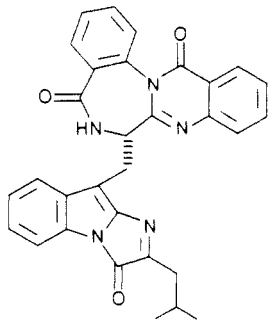
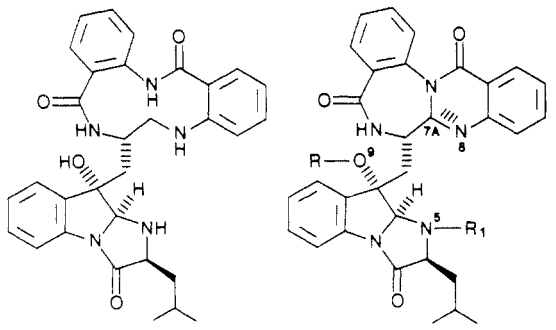


2

and O-methylated products.[8]

Following this rudimentary probe into the chemical reactivity of 1, we began an investigation directed toward selectively modifying the various reactive sites (vide supra). It was discovered that the 7A–8 imine bond of 1 was reduced efficiently and stereoselectively with sodium cyanoborohydride in acetic acid.[9] In this way, the saturated analogue 4 was produced as a single diastereomer in good yield. Further, this reaction could be carried out in the presence of other sensitive functionality (e.g., 12 → 13) or modified, such that 7A–8 imine bond reduction was concurrent with N-5 alkylation. This reductive alkylation procedure efficiently produced compounds 7 and 8. It should be noted that, in the conversion of 1 to 7, the latter compound was the exclusive product; surprisingly under these conditions, none of the cleavage product, the macrocyclic lactam 3, could be detected.[10]



3 4-20

After some experimentation, acylation of the secondary N-5 nitrogen became straightforward. Typically, carboxylic acid anhydrides in the presence of 4-(dimethylamino)pyridine[11] served as electrophilic agents. Following this procedure, the N-5 nitrogen could be acylated without protection of the tertiary hydroxyl group. Succinic anhydride proved to be an exception and both *N*-acyl (**15**) and *O*-acyl (**20**) products were isolated. When these acylations were attempted using carboxylic acids and common peptide coupling reagents (e.g., dicyclohexylcarbodiimide, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, or diphenylphosphoryl azide), the reactions were not reproducible and yields were uniformly low.

The tertiary hydroxyl group in 1 could only be selectively derivatized after the secondary nitrogen (N-5) was protected (cf. entry 6) and deprotected. Nevertheless, attempts to form sulfate and phosphonate esters at this position failed after numerous trials under a variety of reaction conditions.

Selective reaction of the seven-membered ring amide bond of 1 was problematic, and only a few probe experiments were performed. Approaches to the total synthesis of these analogues by a novel route will be the subject of a separate disclosure.

Biology. The structural analogues **4–20** of asperlicin generated in this study were tested as inhibitors of the binding of [^{125}I]CCK to rat pancreas and guinea pig brain receptors. These data are summarized in Table II where they are compared with that of asperlicin (**1**).

A comparison between the results obtained in the pancreas and the brain binding assays reveals that nearly all compounds with pancreatic IC_{50} values[12] less than 100 μM exhibited some degree of selectivity for the peripheral CCK receptor. Interestingly, this selectivity may be reversed in one case studied, i.e. **15**, where incorporation of a succinoyl moiety resulted in higher affinity for the CNS receptor. This selectivity reversal appears to be unique for **15** as other derivatives with hydrophilic substituents, notable **14**, **16**, and **17**, did not show this effect. Our understanding of this selectivity reversal must still be regarded as tenuous, since chemical reduction of the 7A–8 imine bond in **15** to give **16** was sufficient to again alter selectivity [i.e. IC_{50}(pancreas) < IC_{50}(brain)].

In most of the cases that were studied, reduction of the 7A–8 imine bond enhanced the CCK inhibitor potency of the product relative to the unsaturated precursor (cf. **1** and **4**, **12** and **13**, **15** and **16**). The exception was compound **19** in which the tertiary hydroxyl group was functionalized. In addition to compound **4**, other analogues that were more potent than asperlicin **1** were prepared. These contained a saturated 7A–8 imine bond and were alkylated at the five-membered ring nitrogen with an ethyl (for **7**) or a phenpropyl (for **8**) group. However, in this limited study, there appears to be no relationship between CCK inhibitor potency and the lipophilicity of the groups at the ring nitrogen position since acylation with polar, hydrophilic groups provided analogues essentially equipotent with asperlicin (cf. **17**). Similarly, removal of protecting groups on the acyl side chain of **11** afforded the more polar **14** and also resulted in enhanced CCK inhibitor potency.

Assay of compounds **18–20** indicated that the tertiary hydroxyl group appears important for inhibitor activity since its functionalization significantly decreases potency.

The most promising analogue of asperlicin to emerge from this study is compound **17**. Although it proved to be less selective than asperlicin for the peripheral vs. brain CCK receptor, it still retained a significant degree of selectivity and was as potent as asperlicin on the pancreatic CCK receptor. In addition, when administered intravenously (8 mg/kg), **17** caused a significant shift of the dose–response curve for CCK-8 in the in situ guinea pig gallbladder preparation.[6b] Importantly, **17** displayed a marked improvement in aqueous solubility (>1.1 mM) compared to asperlicin (~0.1 μM). Such aqueous solubility eliminates the need for organic solvents and thus greatly facilitates the utility of this class of CCK antagonist in physiological and pharmacological studies.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian EM 390 with tetramethylsilane as an

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(12) IC_{50} value is defined as the concentration of compound necessary to cause 50% displacement of [^{125}I]-CCK binding.

Table I. Physicochemical Data of Asperlicin Analogues

compd	R	R_1	7A–8 bond[a]	yield, %	mp, °C	TLC[b] R_f	formula	anal.[c,d]
4	H	H	S	82	265–267 dec	A (0.48)	$C_{31}H_{31}N_5O_4 \cdot H_2O$	C, H, N
5	H	$-C(=O)CH_3$	U	88	178–180	B (0.72)	$C_{33}H_{31}N_5O_5$	C, H, N
6	H	$-C(=O)OCH_2C_6H_5$	U	88	147	C (0.31)	$C_{39}H_{35}N_5O_6 \cdot 0.5C_2H_6O$	C, H, N
7	H	CH_2CH_3	S	92	179–183	C (0.26)	$C_{33}H_{35}N_5O_4 \cdot 0.25H_2O$	C, H, N
8	H	$CH_2CH_2CH_2C_6H_5$	S	92	156	D (0.33)	$C_{40}H_{41}N_5O_4 \cdot 1.5H_2O$	C, H, N
9	H	$-C(=O)CH(NHBoc)CH_2C_6H_4OCH_2C_6H_5$	U	96	160	E (0.27)	$C_{52}H_{52}N_6O_8$	C, H, N
10	H	$-C(=O)CH(NHBoc)CH_2C_6H_4OH$	U	98	163–169	E (0.13)	$C_{45}H_{46}N_6O_8 \cdot 0.5H_2O$	C, H, N
11	H	$-C(=O)CH(NHBoc)(CH_2)_4NHC(=O)OCH_2C_6H_5$	U	75	138	C (0.37)	$C_{50}H_{55}H_7O_9 \cdot 0.75H_2O$	C, H, N
12	H	$-C(=O)CH(NHBoc)(CH_2)_3NHC(=O)OCH_2C_6H_5$	U	57	79–89[e]	F (0.57)	$C_{49}H_{53}N_7O_9 \cdot 0.75C_4H_8O_2$	C, H, N
13	H	$-C(=O)CH(NHBoc)(CH_2)_3NHC(=O)OCH_2C_6H_5$	S	88	154	F (0.14)	$C_{49}H_{55}H_7O_9 \cdot 1.5H_2O$	C, H, N
14	H	$-C(=O)CH(NH_2)(CH_2)_4NH_2 \cdot 2HCl$	U	85[f]	120 dec	G (0.17)	$C_{37}H_{43}Cl_2N_7O_5 \cdot 3.5H_2O$	C, H, N
15	H	$-C(=O)(CH_2)_2CO_2H$	U	43	200 dec	H (0.29)	$C_{35}H_{33}N_5O_7$	C, H, N
16	H	$-C(=O)(CH_2)_2CO_2H$	S	70	211	I (0.21)	$C_{35}H_{35}N_5O_7 \cdot 2H_2O$	C, H, N[h]
17	H	$-C(=O)(CH_2)_2CO_2Na$	S	100	240 dec	ND[g]	$C_{35}H_{34}N_5O_7Na \cdot 3H_2O$	C, H, N[i]
18	$-C(=O)(CH_2)_2C(=O)OCH_2C_6H_5$	$-C(=O)OCH_2C_6H_5$	U	36	73–83[e]	E (0.56)	$C_{50}H_{45}N_5O_9 \cdot 0.5C_6H_{14}$	C, H, N
19	$-C(=O)(CH_2)_2C(=O)OCH_2C_6H_5$	$-C(=O)OCH_2C_6H_5$	S	53	103–105	J (0.42)	$C_{50}H_{47}N_5O_9 \cdot H_2O$	C, H, N
20	$-C(=O)(CH_2)_2CO_2H$	H	U	26	156–163	H (0.45)	$C_{35}H_{33}N_5O_7 \cdot H_2O$	C, H, N[j]

[a] Key: S = saturated; U = unsaturated. [b] Thin-layer chromatography solvent systems: A = CH_2Cl_2–MeOH, 9:1; B = $CHCl_3$–MeOH, 9:1; C = $CHCl_3$–EtOH, 95:5; D = $CHCl_3$–MeOH, 93:7; E = hexane–EtOAc, 1:1; F = hexane–EtOAc, 3:7; G = $CHCl_3$–EtOH–NH_3, 80:20:2; H = $CHCl_3$–MeOH–HOAc, 93:6:1; I = $CHCl_3$–MeOH–HOAc, 92:7:1; J = $CHCl_3$–EtOH, 97:3. [c] Elemental analyses were within ±0.4% of the theoretical calculated value. [d] The identity of all compounds was further confirmed by FAB mass spectrometry, by IR, and ^1H NMR. [e] Foam. [f] From 11, overall yield, two steps. [g] Not determined. [h] N: calcd, 10.40; found, 10.93. [i] N: calcd, 9.81; found, 10.32. [j] N: calcd, 10.71; found, 10.11.

internal standard or Nicolet NT-360 spectrometer with an internal lock on the deuterium resonance of the solvent. Fast atom bombardment (FAB) mass spectra were run on a Finnigan Mat 731 instrument. Infrared spectra were recorded on a Perkin-Elmer 297 spectrophotometer. HPLC was performed on a Hewlett-Packard 1084B instrument. Flash chromatography was performed as described by Still[13] on silica gel (E. Merck, 0.04–0.063 mm); thin-layer chromatography (TLC) and preparative thick-layer chromatography (PLC) were carried out on E. Merck 60F-254 precoated silica gel plates (0.25, 0.5, 2.0 mm).

General Procedure for the Reduction of the 7A–8 Imine Bond of Compounds 4, 13, 16, 17, and 19. Preparation of [2*S*-[2α,9β,9(7*R,7a*R**),9αβ]]-6,7,7a,8-Tetrahydro-7-[[2,3,9,9a-tetrahydro-9-hydroxy-2-(2-methylpropyl)-3-oxo-1*H*-imidazo[1,2-*a*]indol-9-yl]methyl]quinazolino[3,2-*a*]-[1,4]benzodiazepine-5,13-dione (4).** Sodium cyanoborohydride (0.41 g, 6.44 mmol) was added in one portion to a magnetically stirred solution of 12 mL glacial acetic acid containing asperlicin (1; 1.0 g, 1.87 mmol), at 12 °C. The reaction mixture was stirred for 15 min, diluted with 12 mL of water, and rendered alkaline with saturated sodium carbonate solution. The resulting precipitate was collected, washed with water, and dried to give the title compound. Additional purification could be carried out by either trituration or recrystallization from appropriate solvents.

General Procedure for the N-Acylation of Asperlicin (1). Preparation of Compounds 5, 9, 11, 12, and 15. To a solution of asperlicin (1; 2.5 mmol) in 25 mL of methylene chloride was added the corresponding symmetrical acid anhydride[14] (12.5 mmol) or mixed anhydride (prepared with isobutyl chloroformate and *N*-methylmorpholine)[15] and 4-(dimethylamino)pyridine (2.5 mmol). The resulting reaction mixture was protected from moisture and stirred at room temperature (20 h). The reaction

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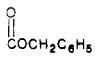
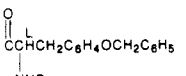
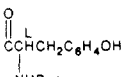
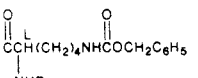
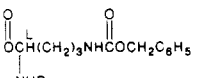
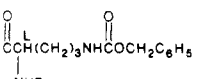
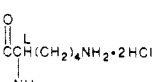
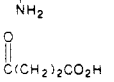
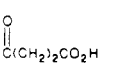
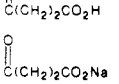
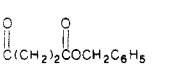
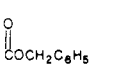
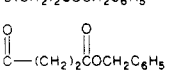
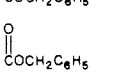
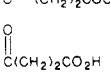
Table II. Effect of CCK Antagonists on [^{125}I]CCK-33 Binding in Pancreas and Brain

compd	R	R_1	7A–8 bond[a]	[^{125}I]CCK-33 binding IC_{50}, μM	
				pancreas	brain
1	H	H	U	1.4	>100[b]
4	H	H	S	0.2	>100 (289)[c]
5	H		U	22	>100 (>300)
6	H		U	5	>100
7	H	CH_2CH_3	S	0.4	81
8	H	$CH_2CH_2CH_2C_6H_5$	S	0.2	>100
9	H		U	>100	>100
10	H		U	46	>100
11	H		U	>100	>100
12	H		U	4.3	>100 (>300)
13	H		S	1.3	>100
14	H		U	2.5	>100 (318)
15	H		U	>100 (262)	81
16	H		S	4.3	54
17	H		S	1.0	40
18			U	100	100
19			S	100	>100
20		H	U	85	>100

[a] Key: U = unsaturated; S = saturated. [b] The value >100 indicates that less than 50% inhibition was observed at 100 μM, the highest concentration tested. [c] Values in parentheses represent the results of additional assays using higher concentrations of the test compounds.

mixture was diluted to five times the original volume with methylene chloride and then washed with 10% citric acid solution and brine. Concentration of the dried (sodium sulfate) organic extracts afforded the product that was purified by recrystallization and/or silica gel chromatography to give the analytical sample.

Synthesis of Phenylmethyl 2,3,9,9a-Tetrahydro-9-hydroxy-2-(2-methylpropyl)-3-oxo-9-[(5,6,7,13-tetrahydro-5,13-dioxoquinazolino[3,2-*a*][1,4]benzodiazepin-7-yl)-methyl]-1*H*-imidazo[1,2-*a*]indole-1-carboxylic Acid Ester (6). This compound was prepared by the same method as for the preparation of 5, except that the acylating agent was a chloro carbonate.

Preparation of 3-Dioxo-9-[(5,6,7,7a,8,13-hexahydro-5,13-dioxoquinazolino[3,2-*a*][1,4]benzodiazepin-7-yl)methyl]-2,3,9,9a-tetrahydro-9-hydroxy-2-(2-methylpropyl)-γ-1*H*-imidazo[1,2-*a*]indole-1-butanoic Acid, Sodium Salt (17). A 0.05 M solution of sodium hydroxide (1.8 mL) was added to 0.09 mmol of acid 16. After stirring for 15 min at 23 °C, the homogeneous reaction mixture was concentrated to give the title compound.

Preparation of 1,1-Dimethylethyl [1-[(4-Hydroxyphenyl)methyl]-2-oxo-2-[2,3,9,9a-tetrahydro-9-hydroxy-2-(2-methylpropyl)-3-oxo-9-[(5,6,7,13-tetrahydro-5,13-dioxoquinazolino[3,2-*a*][1,4]benzodiazepin-7-yl)methyl]-1*H*-imidazo[1,2-*a*]indol-1-yl]ethyl]carbonic Acid Ester (10). This material was synthesized by mixing 650 mg (0.73 mmol) of 9 with 300 mg of 10% palladium/carbon catalyst in 30 mL of ethanol and hydrogenating the resulting suspension at 48 psi and 23 °C for 4.5 h. Flash chromatography on silica gel (hexane–ethyl acetate, 45:55) afforded the analytical sample.

Preparation of 1-(2,6-Diamino-1-oxohexyl)-1,2,9,9a-tetrahydro-9-hydroxy-2-(2-methylpropyl)-9-[(5,6,7,13-tetrahydro-5,13-dioxoquinazolino[3,2-*a*][1,4]benzodiazepin-7-yl)methyl]-3*H*-imidazo[1,2-*a*]indol-3-one Dihydrochloride (14). Compound 11 (800 mg, 0.89 mmol) was dissolved in 40 mL of a mixture of ethanol–50% aqueous acetic acid (4:1, v/v), treated with 300 mg of 10% palladium/carbon catalyst, and hydrogenated at 48 psi and 23 °C for 2.5 h. The reaction mixture was filtered through Celite and concentrated. The residual oil was chromatographed on silica gel with, initially, chloroform–ethanol–concentrated ammonium hydroxide (80:10:1); after forty 20-mL fractions, the gradient was changed to 80:20:2. The intermediate product was contained in fractions 51–66. The pooled fractions were concentrated under reduced pressure, and the remaining oil (625 mg) was dissolved in 25 mL of ethyl acetate. This solution was cooled to 0 °C and treated with a continuous stream of

hydrogen chloride gas for 1 h. Excess reagent and solvent were rotoevaporated to give the desired product as a white powder.

Reductive Alkylation of Asperlicin (1). Preparation of 7-[[1-Ethyl-2,3,9,9a-tetrahydro-9-hydroxy-2-(2-methylpropyl)-3-oxo-1*H*-imidazo[1,2-*a*]indol-9-yl]methyl]-6,7,7a,8-tetrahydroquinazolino[3,2-*a*][1,4]benzodiazepine-5,13-dione (7). Sodium cyanoborohydride (158 mg, 2.52 mmol) was added at room temperature to a solution of 10 mL of glacial acetic acid containing 450 mg (0.84 mmol) of 1. After 2 h, 3 equiv more of sodium cyanoborohydride was added and the reaction mixture was stirred at 55 °C for 14 h. The reaction mixture was cooled and poured into 150 mL of water. The resulting precipitate was collected, washed with water, and dried. The analytical sample was obtained as a white solid via silica gel chromatography ($CHCl_3$–EtOH, 95:5) followed by trituration with ether.

Preparation of 6,7,7a,8-Tetrahydro-7-[[2,3,9,9a-tetrahydro-9-hydroxy-2-(2-methylpropyl)-3-oxo-1-(3-phenylpropyl)imidazo[1,2-*a*]indol-9-yl]methyl]quinazolino[3,2-*a*][1,4]benzodiazepine-5,13-dione (8). Asperlicin (1; 535 mg, 1.0 mmol) was dissolved in 10 mL of glacial acetic acid. 3-Phenylpropionaldehyde (400 mg, 3.0 mmol) and sodium cyanoborohydride (504 mg, 8.0 mmol) were then added to this mixture. The reaction was stirred at room temperature for 60 h and worked up as in the preparation of 7.

Acylation of the Tertiary Hydroxyl Group. Preparation of Phenylmethyl [2,3,9,9a-Tetrahydro-2-(2-methylpropyl)-3-oxo-1-[(phenylmethoxy)carbonyl]-9-[(5,6,7,13-tetrahydro-5,13-dioxoquinazolino[3,2-*a*][1,4]benzodiazepin-7-yl)-methyl]-1*H*-imidazo[1,2-*a*]indol-9-yl]butanedioic Acid Ester (18). To 1.53 g (2.3 mmol) of 6 in 10 mL of methylene chloride was added in succession benzyl succinate half-acid ester (718 mg, 3.45 mmol), 4-(dimethylamino)pyridine[11] (421 mg, 3.45 mmol), and a 1 M solution of dicyclohexylcarbodiimide in methylene chloride (3.45 mL, 3.45 mmol). The reaction mixture was protected from moisture and stirred at room temperature overnight. The reaction mixture was filtered, diluted to 250 mL with methylene chloride, and washed with 5% citric acid solution, 50% sodium bicarbonate solution, and brine. The dried ($MgSO_4$) organic phase was rotoevaporated to give an amorphous solid that was chromatographed on silica gel (hexane–ethyl acetate, 7:3) to yield the analytical sample.

Preparation of Mono[2,3,9,9a-Tetrahydro-2-(2-methylpropyl)-3-oxo-9-[(5,6,7,13-tetrahydro-5,13-dioxoquinazolino-[3,2-*a*][1,4]benzodizaepin-7-yl)methyl]-1*H*-imidazo[1,2-*a*]-indol-9-yl] Butanedioic Acid Ester (20). A solution of 18 (300 mg, 0.35 mmol) in 75 mL of ethanol was treated with 50 mg of 10% palladium/carbon catalyst and hydrogenated at 50 psi at 23 °C for 4.5 h. Catalyst and solvent were removed to give an oil that was purified by PLC ($CHCl_3$–EtOH, 9:1) to afford the analytical sample as an off-white solid.

Cholecystokinin Receptor Binding Method. Pancreas. CCK-33 was radiolabeled with ^{125}I Bolton Hunter reagent (2000 Ci/mmol) as described by Sankaran.[16] Rat pancreatic receptor binding was performed according to Innis and Snyder[17] with the minor modification of adding the additional protease inhibitors, (phenylmethyl)sulfonyl fluoride and *o*-phenanthroline, which have no effect on the [^{125}I]-CCK receptor binding assay.

Brain. CCK-33 was radiolabeled and the binding to guinea pig cortical membranes was performed according to Saito.[18] In both pancreas and brain binding assays several concentrations of the test compounds were examined in triplicate and IC_{50} values determined by regression analysis.

Acknowledgment. It is a pleasure to acknowledge the assistance of S. L. Fitzpatrick, J.-P. Moreau, J. S. Murphy, and J. Smith for analytical support. We are indebted to Dr. M. A. Goetz for generously supplying asperlicin. We thank Drs. G. D. Hartman and R. Pendleton for useful discussions, Drs. P. S. Anderson and B. V. Clineschmidt for support and encouragement, and M. Z. Banker for preparing the manuscript.

Registry No. 1, 93413-04-8; 4, 103303-31-7; 5, 102743-49-7; 6, 102743-51-1; 7, 103303-32-8; 8, 102743-52-2; 9, 103241-31-2; 10, 103241-32-3; 11, 102743-57-7; 12, 102743-56-6; 13, 103022-89-5; 14, 102996-16-7; 14 (free base), 103303-33-9; 15, 102996-15-6; 16, 102996-17-8; 17, 102996-18-9; 18, 103241-33-4; 19, 103241-34-5; 20, 102996-22-5; (L)-*p*-$HO_2CCH(NHBoc)CH_2C_6H_4OCH_2Ph$, 2130-96-3; (L)-$HO_2CCH(NHBoc)(CH_2)_4NHCO_2CH_2Ph$, 2389-45-9; (L)-$HO_2CCH(NHBoc)(CH_2)_3NHCO_2CH_2Ph$, 2480-93-5; 3-phenyl propanal, 104-53-0; monobenzyl succinate, 103-40-2; cholecystokinin, 9011-97-6.

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Cardiac Glycosides. 7. Sugar Stereochemistry and Cardiac Glycoside Activity

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Digitoxigenin α-L-, β-L-, α-D-, and β-D-glucosides; α-L-, β-L-, α-D-, and β-D-mannosides; and α-L- and β-L-rhamnosides were stereoselectively synthesized from the corresponding sugar tetrabenzyl trichloroacetimidates. The Na^+,K^+-ATPase receptor inhibitory activities of these glycosides (as a measure of receptor binding) were compared with those of digitoxigenin, digitoxigenin 6′-hydroxy-β-D-digitoxoside, digitoxigenin β-D-galactoside, and digitoxigenin β-D-digitoxoside. The observed activities reveal that a given sugar substituent may have a role in binding of some glycoside stereoisomers, but not others. With α-L- and possibly β-L-rhamnosides, the 5′-CH_3 and 4′-OH appear to have a predominant role in binding to the Na^+,K^+-ATPase receptor. Addition of a 6′-OH to form the corresponding mannosides dramatically disrupts the effect of both the 5′-CH_3 and 4′-OH in prompting receptor binding of the α-L isomer. However, with the β-L isomer, some influence of 4′-OH, 3′-OH, and 2′-OH binding remains. With β-D-glycosides, binding via the "5′-CH_3 site" appears to be of little importance and addition of a 6′-OH diminishes activity only slightly. With these β-D-glycosides, an equatorial 4′-OH, axial 3′-OH, and equatorial 2′-OH groups appear to contribute to binding.

Digitoxin, digoxin, and most other naturally occurring active cardiac glycosides have sugars with β-D stereochemistry. The notable exception is ouabain, an α-L-rhamnoside. In spite of this remarkably different stereochemistry, ouabain is very potent. Thus, there has been considerable interest in trying to understand the stereochemical preferences of the sugar binding site on the cardiac glycoside receptor Na^+,K^+-ATPase.[1-6] Brown and

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0022-2623/86/1829-1945$01.50/0