Marshall reagent is DDS. Calculations based on other studies[14] have shown that blood levels of 2-3 μg/ml of DDS are required to prevent the development of *P. berghei* infection in mice. TAHDS showed significant antimalarial activity at 40 mg/kg ip; based on the methemoglobin studies, a maximum of 10% of that absorbed might be present as DDS. It seems unlikely that levels of 2-3 μg/ml would be achieved after administration of 40 mg/kg of a compound of such poor solubility and low possible DDS yield *in vivo*.

TAHDS was tested against two other parasites. It showed little activity against *Trichinella spiralis* in mice (at 25 mg/kg orally plus 25 mg/kg sc) or against *Eimeria tenella* in chicks fed diets containing 0.05% TAHDS for 2 days prior to and 6 days after infection.

In mice, TAHDS showed antileprotic activity against *Mycobacterium leprae* approaching that of DDS. It had no activity against a variety of tumors.

TAHDS produced no overt effects or signs of toxicity in any of the mouse tests or when administered orally to rats at a dose of 200 mg/kg. It did not exacerbate adjuvant-induced arthritis in rats or inhibit gastric acid secretion in gastric fistula rats at a dose of 50 mg/kg po; 24 hr after administration of 80 mg/kg po to chronic, metacorticoid-hypertensive rats, there was a significant reduction in systolic blood pressure.

Experimental Section‡

4,4′-Dihydroxylaminodiphenyl Sulfone (DHDS). Bis(*p*-nitrophenyl) sulfone[15] (54 g, 0.18 mol) was suspended in 1.2 l. of 80% EtOH containing several milliliters of AcOH. Ammonium chloride (90 g) and Zn dust (65 g) were added and the mixture was heated at reflux with stirring for 45 min. The resulting pale yellow solution was filtered, the filtrate was concentrated to 150 ml, and 700 ml of H_2O preheated to 95° was added to this warm solution. A small amount of orange gum formed after 2 or 3 min and the mixture was then immediately filtered. After 5 min, a crystalline solid separated from the filtrate. The solution was rapidly chilled and filtered, giving 32 g of light yellow needles, mp 160–165° (170° dec). This crude DHDS was suitable for use in the acetylation reaction. DHDS is unstable in aqueous solution, being converted to a very high-melting (greater than 300°) mixture of solids. Further purification is tedious and difficult to reproduce, but 1-2-g amounts could be purified as follows. The crude hydroxylamine was dissolved in a minute quantity of preheated (95°) H_2O and filtered by suction 3–4 times as the solution slowly cooled. When the first crystals started to form, the solution was rapidly chilled and the crystalline solid was immediately filtered. After three recrystallizations, the crystals were obtained as colorless needles, mp 184–186° dec (lit.[10] mp 170° dec). *Anal.* ($C_{12}H_{12}N_2O_4S$) C, H, S. When this material is slowly heated, left in aqueous solution for a lengthy period, or treated with acids or bases, it is converted to a mixture of high-melting solids whose nature has not been determined.

4,4′-Bis(*N,O*-diacetylhydroxylamino)diphenyl Sulfone (TAHDS). A suspension of 30 g of crude DHDS in 400 ml of Ac_2O was heated on a steam bath. After 15 min all solids dissolved giving a clear orange solution. Excess Ac_2O was removed *in vacuo*, EtOH was added to the residue, and the suspension was again concentrated *in vacuo* giving an orange slush. This was triturated with 150 ml of EtOH; then 600 ml of H_2O was added and the resulting gummy solid was filtered (60 g), mp 167–172°. The gum was triturated with $CHCl_3$ and filtered. The insoluble solid, 3 g, mp 260–265°, was discarded (probably DADS). The filtrate was dried ($MgSO_4$) and then concentrated to give 34 g of orange oil that crystallized on cooling. This was recrystallized from EtOH and then three times from $CHCl_3$–hexane to give 13 g of white solid, mp 186–188°. Concentration of the filtrate gave another 10 g, mp 179–183°. *Anal.* ($C_{20}H_{20}N_2O_8S$) C, H, N, S. The solubility of TAHDS is low (<2.5 γ/ml in H_2O, <100 γ/ml in EtOH); however, 120 mg/ml may be dissolved in DMSO. In the search for a convenient assay for THDS that could be used with plasma, TAHDS was heated with Zn and HCl at 100°. The products did not contain DDS (as shown by tlc) nor did they give a positive color reaction for arylamines with the Bratton–Marshall reagent.[13]

‡Melting points are corrected; boiling points are uncorrected. Elemental analyses were performed by Miss M. Carroll and coworkers of the Analytical and Physical Chemistry Section, Smith Kline & French Laboratories. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

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L-4′-Cyano-3-(2,2,2-trifluoroacetamido)succinanilic Acid and Related Synthetic Sweetening Agents

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Since the discovery of saccharin in 1879[1] a number of sweetening agents have been described.[2-5] These compounds are all of interest, since they reflect the diversity of chemical structure and the complete lack of any unifying generalization that would aid in predicting sweetness.

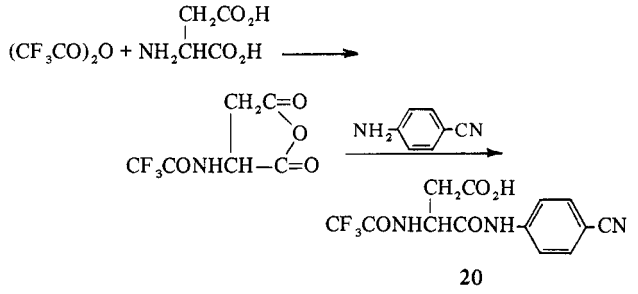
In 1968 the pronounced sucrose-like taste of L-aspartyl-L-phenylalanine methyl ester (**43**) was reported.[6] Although no other compound more patently sweet than the initial discovery was found, the retention of sweetness was noted to be correlated with both the unsubstituted amino and β-carboxy groups of aspartic acid. L-Aspartic acid could not be replaced without loss of sweetening activity, but con-

siderable modification of the phenylalanine portion was tolerated.[7,8]

The accidental tasting of L-3-(2,2,2-trifluoroacetamido)-succinanilic acid (**1**) by one of the authors (M. L.) resulted in the discovery of sweetness. From this chance encounter we decided to synthesize *N*-acyl-L-aspartyl-α-anilides and -α-amides to evaluate the importance of a number of acyl groups such as trichloroacetyl, monochloroacetyl, dichloroacetyl, chlorodifluoroacetyl, and acetyl for their contribution toward activity.

Compounds were synthesized by a method first proposed by Weygand for the synthesis of *N*-trifluoroacetyl-L-aspartyl-α-anilides[9] and *N*-trifluoroacetyl-L-aspartyl-α-peptides.[10] Although the β isomer is a distinct alternative structure for the aminolysis of *N*-trifluoroacetyl-L-aspartic acid anhydride, this possibility may essentially be ruled out, since Weygand showed that the inductive power of the trifluoroacetyl group directs the attack of the nucleophilic amine exclusively to the α position of the anhydride (Scheme I).

Scheme I



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The quantitative evaluation of the degree of sweetness was based on comparing the taste of test solutions with a standard 0.06 *M* solution of sucrose. All solutions were adjusted to pH 7. Test solutions were tasted at increasingly greater dilutions until a potency was found which matched the taste of the standard sucrose solution. Sweetness was then expressed as the ratio of the molar concentration of the test sample to that of the standard.

From the *N*-trifluoroacetyl-L-aspartyl-α-anilides (Table I) synthesized, a number of interesting structure-sweetness correlations were made. Compared with **1**, the lack of sweetness of **2**, synthesized from D-aspartic acid, indicated that the L configuration was a requirement for sweetness. All subsequent syntheses were based on L-aspartic acid.

Of the *N*-trifluoroacetyl-L-aspartyl-α-amides synthesized (**30–40**), none were found to be sweet.

N-Trifluoroacetyl-L-aspartyl-α-anilide (**1**), as well as the *p*-fluoro (**5**), *p*-chloro (**12**), *p*-bromo (**18**), and *p*-cyano (**20**) anilides, was sweet. L-4′-Cyano-3-(2,2,2-trifluoroacetamido)-succinanilic acid (**20**) was found to have a very pleasant taste and to be the most potent sweetening agent, it being approximately 3000 times as sweet as sucrose. In this series of compounds, the *p*-iodo (**19**), *p*-hydroxy (**24**), *p*-methyl (**25**), *p*-trifluoromethyl (**26**), *p*-acetamido (**27**), *p*-sulfonamido (**28**), and *p*-carboxethyl (**29**) anilides were devoid of sweetness. Furthermore, the *o*-fluoro (**11**), *o,p*-difluoro (**10**), *o,m*-difluoro (**9**), *o*-chloro (**16**), and *o*-carboxy, *p*-chloro (**17**) anilides were not sweet.

The replacement of the trifluoroacetyl group of the most potent sweetening agent (**20**) with the trichloroacetyl group (**21**) resulted in no loss in the degree of sweetness. A similar relationship was found to hold for compounds **1** and **3**. Replacement with chlorodifluoroacetyl (**4, 6, 13**), dichloroacetyl (**7**), monochloroacetyl (**8**), and acetyl (**14, 22**) resulted in compounds not only lacking in sweetness but also having an unpleasant sour taste.

To evaluate the contribution of the trifluoroacetyl and trichloroacetyl groups to the degree of sweetness, the acyl groups were removed from **12** and **20** by basic hydrolysis. This resulted in compounds **15** and **23**, which were only slightly sweet. Evidently the acyl group makes a major contribution to the sweetness of the *N*-acyl-L-aspartyl-α-anilides **12** and **20**. The *N*-trifluoroacetyl-L-aspartyl-L-phenylalanine methyl ester (**42**) was prepared by the method of Weygand[10] and its degree of sweetness was compared with that of L-aspartyl-L-phenylalanine methyl ester (**43**). The latter ester was prepared by the active ester method,[11] using protecting groups for the amino and β-carboxy groups that are easily removed by hydrogenolysis with Pd/C. Very little difference in sweetness was detected. Substitution of the free amino group of the dipeptide ester **43** with the trifluoroacetyl group did not enhance the sweetness. No generalization can be drawn from this example at this time, since we have only a limited number of peptides to draw on for structure-activity correlations.

The trifluoroacetyl and trichloroacetyl groups were the only acyl groups found that enhanced the characteristic sweetness of the L-aspartyl-α-anilides. This series of anilides further extends our knowledge of sweetners synthesized from L-aspartic acid but does not rule out further synthetic developments.

Experimental Section†

***N*-Trifluoroacetyl-L-aspartic Acid Anhydride.** To L-aspartic acid (212.8 g, 1.6 mol), cooled in an acetone–Dry Ice bath, was slowly added with stirring trifluoroacetic acid anhydride (840 g, 4 mol). The bath was removed and the reaction allowed to warm up. After the vigorous exothermic reaction had subsided, refluxing was continued for 2 hr. When cool, the mixture was poured into 1 l. of dry petroleum ether. The resulting solid was collected on a sintered glass Büchner funnel and washed with 2 l. of petroleum ether and 500 ml of Et_2O. Drying overnight *in vacuo* at 40° yielded 338 g (98%) of product, mp 134–135°. *Anal.* ($C_6H_4NO_4F_3$) C, H, N.

***N*-Trichloroacetyl-L-aspartic Acid Anhydride.** L-Aspartic acid (66.5 g, 0.5 mol) was heated with trichloroacetic acid anhydride (300 g, 1.15 mol) at 85° for 2 hr. When cool, the mixture was triturated with ether and the solids were collected. The product was recrystallized by dissolving in acetone–ether (1:5) and adding petroleum ether. Drying *in vacuo* at room temperature yielded 31.7 g (24%) of product, mp 167–168°. *Anal.* Calcd for $C_6H_4NO_4Cl_3$: C, 27.67; H, 1.55; Cl, 40.84. Found: C, 28.51; H, 1.67; Cl, 38.91.

***N*-Chloroacetyl-L-aspartic Acid Anhydride.** L-Aspartic acid (19.9 g, 0.15 mol) was heated with chloroacetic acid anhydride (80 g, 0.46 mol) in 200 ml of THF at 70° for 2 hr. After the mixture had stood overnight at room temperature the THF was removed in a rotary evaporator and the solids were washed with Et_2O and collected. The product, after drying *in vacuo* at room temperature, amounted to 27 g (94%), mp 165–169°. *Anal.* ($C_6H_6NO_4Cl$) C, H, N.

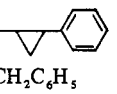
***N*-Chlorodifluoroacetyl-L-aspartic Acid Anhydride.** L-Aspartic acid (38 g, 0.28 mol) was heated with chlorodifluoroacetic acid anhydride (200 g, 0.83 mol) for 1 hr at 104°. After standing overnight at room temperature, the solids were washed with petroleum ether and collected. Drying *in vacuo* at 40° yielded 60 g (93.9%) of product, mp 152–154°. *Anal.* ($C_6H_4NO_4ClF_2$) C, H, N.

***N*-Acetyl-L-aspartic Acid Anhydride.** L-Aspartic acid (60 g, 0.45 mol) was combined with 60 ml of 30% HBr–AcOH. The combination was allowed to stand for 30 min, 420 ml of Ac_2O

†All melting points (uncorrected) were determined with a Thomas-Hoover melting point apparatus. Microanalyses were performed by and ir spectra were obtained under the direction of Mr. B. Hofmann. Where analyses are indicated only by symbols of the elements, analytical results for these elements were within ±0.4% of the theoretical values. Otherwise, the results for C, H, and N are given.

Table I. *N*-Acyl-L-aspartyl-α-amides and -α-anilides

$$\begin{array}{l} CH_2CO_2H \\ | \\ Z\text{-}NHCHCO\text{-}X \end{array}$$

Compd	Z	X	Taste[a]	Recrystn solvent[b]	Yield, %	Mp,[c] °C	Formula[d]
1	CF_3CO	HNC_6H_5	12	A, W	46	197–198	$C_{12}H_{11}F_3N_2O_4$
2[f]	CF_3CO	HNC_6H_5	–	M, W	26	170–173	$C_{12}H_{11}F_3N_2O_4$[e]
3	CCl_3CO	HNC_6H_5	+	Et, PE	18	230–231	$C_{12}H_{11}Cl_3N_2O_4$[e]
4	$CClF_2CO$	HNC_6H_5	–	M	56	201–202	$C_{24}H_{34}ClF_2N_3O_4$[g]
5	CF_3CO	HN-4-F-C_6H_4	+	M, IE	33	218 dec	$C_{24}H_{33}F_4N_3O_4$[g]
6	$CClF_2CO$	HN-4-F-C_6H_4	–	M, IE	53	199–201	$C_{24}H_{33}ClF_3N_3O_4$[g]
7	$CHCl_2CO$	HN-4-F-C_6H_4	–	M, IE	84	281–284	$C_{24}H_{34}Cl_2FN_3O_4$[g]
8	CH_2ClCO	HN-4-F-C_6H_4	–	M	55	209–212	$C_{24}H_{35}ClFN_3O_4$[g]
9	CF_3CO	HN-2,5-F-C_6H_3	–	M, IE	48	202–203	$C_{24}H_{32}F_5N_3O_4$[g]
10	CF_3CO	HN-2,4-F-C_6H_3	–	M, IE	66	207–208	$C_{24}H_{32}F_5N_3O_4$[g]
11	CF_3CO	HN-2-F-C_6H_4	–	M, IE	57	205–206	$C_{24}H_{33}F_4N_3O_4$[g]
12	CF_3CO	HN-4-Cl-C_6H_4	120	AN	39	190–191.5	$C_{12}H_{10}ClF_3N_2O_4$
13	$CClF_2CO$	HN-4-Cl-C_6H_4	–	M	44	206–208	$C_{24}H_{33}Cl_2F_2N_3O_4$[g]
14	CH_3CO	HN-4-Cl-C_6H_4	–	AN	21	207–208	$C_{12}H_{13}ClN_2O_4$
15	H	HN-4-Cl-C_6H_4	12	W	38	229 dec	$C_{10}H_{11}ClN_2O_3$
16	CF_3CO	HN-2-Cl-C_6H_4	–	M, IE	23	199–201	$C_{24}H_{33}ClF_3N_3O_4$[g]
17	CF_3CO	HN-2-CO_2H-4-Cl-C_6H_3	–	M, IE	51	210–211	$C_{37}H_{56}ClF_3N_4O_6$[g]
18	CF_3CO	HN-4-Br-C_6H_4	120	AN	47	200–202	$C_{12}H_{10}BrF_3N_2O_4$
19	CF_3CO	HN-4-I-C_6H_4	–	AN	69	214 dec	$C_{12}H_{10}F_3IN_2O_4$
20	CF_3CO	HN-4-CN-C_6H_4	3000	AN	62	187–188	$C_{13}H_{10}F_3N_3O_4$
21	CCl_3CO	HN-4-CN-C_6H_4	3000[i]	M, IE	32	226 dec	$C_{26}H_{20}CaCl_6N_6O_8$[j]
22	CH_3CO	HN-4-CN-C_6H_4	–	AN	26	210–211	$C_{13}H_{13}N_3O_4$[e]
23	H	HN-4-CN-C_6H_4	12	B	95	215 dec	$C_{11}H_{11}N_3O_3$
24	CF_3CO	HN-4-OH-C_6H_4	–	AN	57	173 dec	$C_{12}H_{11}F_3N_2O_5$[e]
25	CF_3CO	HN-4-CH_3-C_6H_4	–	A, W	21	162–166	$C_{13}H_{13}F_3N_2O_4$[e]
26	CF_3CO	HN-4-CF_3-C_6H_4	–	AN	67	189–191	$C_{13}H_{10}F_6N_2O_4$[e]
27	CF_3CO	HN-4-$CONH_2$-C_6H_4	–	AN	34	210 dec	$C_{13}H_{12}F_3N_3O_5$
28	CF_3CO	HN-4-SO_2NH_2-C_6H_4	–	T, IE	48	205 dec	$C_{12}H_{12}F_3N_3O_6S$
29	CF_3CO	HN-4-$CO_2C_2H_5$-C_6H_4	–	T, IE	48	185 dec	$C_{27}H_{38}F_3N_3O_6$[g]
30	CF_3CO	HN-c-$C_5H_{10}N$	–	M, IE	31	225 dec	$C_{23}H_{39}F_3N_4O_4$[g]
31	CF_3CO	HN-c-C_6H_{11}	–	T	20	Dec	$C_{24}H_{40}F_3N_3O_4$[g]
32	CF_3CO	HN-c-C_5H_9	–	M, IE	23	230 dec	$C_{23}H_{38}F_3N_3O_4$[g]
33	CF_3CO	HNH	–	W	19	184–187	$C_6H_7F_3N_2O_4$
34	CF_3CO	HN-c-C_3H_5	–	M, IE	36	207–208	$C_{21}H_{34}F_3N_3O_4$[g]
35[h]	CF_3CO	$HNCH(CH_3)CH_2C_6H_5$	–	AN	23	175–177.5	$C_{15}H_{17}F_3N_2O_4$
36	CF_3CO	$HN(CH_2)_5CH_3$	–	AN	39	129–131	$C_{12}H_{19}F_3N_2O_4$
37	CF_3CO	$HNCH(CH_3)(CH_2)_5CH_3$	–	M, IE	47	157–159	$C_{26}H_{46}F_3N_3O_4$[g]
38	CF_3CO	$HN(CH_2)_2$-C_6H_4-OH	–	E	22	188–190	$C_{26}H_{38}F_3N_3O_5$[g]
39	CF_3CO		–	M, IE	36	169–170	$C_{27}H_{38}F_3N_3O_4$[g]
40	CF_3CO	$HNCH_2C_6H_5$	–	E	48	201–202	$C_{25}H_{36}F_3N_3O_4$[g]
41	CF_3CO		–	EA	21	182–185	$C_{28}H_{44}F_3N_3O_4$[g]
42	CF_3CO	$HNCH(CO_2CH_3)CH_2C_6H_5$	120	AN	53	150–152	$C_{16}H_{17}F_3N_2O_6$
43	H	$HNCH(CO_2CH_3)CH_2C_6H_5$	150	W	70	248–249	$C_{14}H_{18}N_2O_5$

[a]Number times sweeter than sugar. In addition, + = sweet, – = not sweet. Based on the taste evaluation of the authors. [b]Solvent abbreviations: A, Me_2CO; M, MeOH; W, H_2O; Et, Et_2O; PE, petroleum ether; IE, *i*-Pr_2O; AN, MeCN; B, *n*-BuOH; T, THF; E, EtOH; EA, EtOAc. [c]Uncorrected. [d]All compounds except those indicated otherwise[e] gave values for C, H, and N within ±0.4% theory. [e]**2**, C: calcd, 47.38; found, 48.94; H: calcd, 3.64; found, 3.81. N: calcd, 9.20; found, 9.81. **3**, C: calcd, 40.77; found, 41.89. H: calcd, 3.13; found, 3.33. N: calcd, 7.92; found, 7.79. **22**, C: calcd, 56.73; found, 56.10. H: calcd, 4.76; found, 4.72. N: calcd, 15.27; found, 15.19. **24**, C: calcd, 45.01; found, 45.54. H: calcd, 3.46; found, 3.81. N: calcd, 8.74; found, 8.45. **25**, C: calcd, 49.05; found, 49.88. H: calcd, 4.11; found, 4.28. N: calcd, 8.80; found, 9.03. **26**, C: calcd, 41.95; found, 41.41. H: calcd, 2.71; found, 2.76. N: calcd, 7.53; found, 7.55. [f]Derived from D-aspartic acid. [g]Dicyclohexylamine salt, prepared in methanol and recrystallized. [h]Derived from *d*-α-methylphenethylamine. [i]Sweetness based on one-half the molecular weight of the calcium salt. [j]Calcium salt, prepared by adding calcium acetate to the derivative in aqueous methanol and recrystallizing.

was added, and the mixture was heated at 60° until clear. The solvent was removed in a rotary evaporator and the residue was triturated with ether. The solid was collected and crystallized from Ac_2O. Drying of the product overnight *in vacuo* at 40° yielded 40 g (56.4%) of product, mp 112–113°. *Anal.* Calcd for $C_6H_7NO_4$: C, 45.85; H, 4.49; N, 8.94. Found: C, 46.92; H, 4.65; N, 7.83.

***N*-Dichloroacetyl-L-aspartic Acid Anhydride.** L-Aspartic acid (18.6 g, 0.14 mol) was heated with dichloroacetic acid anhydride (100 g, 0.41 mol) in 200 ml of acetone under reflux for 1 hr. The acetone was removed in a rotary evaporator and the solids were washed with petroleum ether and collected. The product was recrystallized by dissolving in acetone–ether (1:5) and adding petroleum ether. Drying *in vacuo* at room temperature yielded 24 g (75.7%) of product, mp 150–154°. *Anal.* ($C_6H_5NO_4Cl_2$) C, H, N.

L-4′-Cyano-3-(2,2,2-trifluoroacetamido)succinanilic Acid (20). *N*-Trifluoroacetyl-L-aspartic acid anhydride (42.2 g, 0.2 mol) and 4-cyanoaniline (23.6 g, 0.2 mol) were combined in 200 ml of dry THF and allowed to stand 5 days at room temperature. The solvent was removed in a rotary evaporator and the solids were crystallized from acetonitrile. Drying *in vacuo* at 40° yielded 26.5 g (40.2%) of product, mp 187–188°. *Anal.* ($C_{13}H_{10}N_3O_4F_3$) C, H, N.

The above procedure was used for the synthesis of all *N*-acyl-L-aspartic acid α-amides and α-anilides in Table I (**1–42**).

L-3-Amino-4′-cyanosuccinanilic Acid (23). Compound **20** (9 g, 0.027 mol) was dissolved in 100 ml of 7.4 *M* ammonium hydroxide. The reaction mixture was quickly heated to 85° and the temperature was maintained at this level for 5 min. The mixture was then cooled to room temperature and the water was removed in a rotary

evaporator. The resulting white crystalline solid was recrystallized from 1-butanol. Drying *in vacuo* at 40° yielded 6 g (95.1%) of product, mp 215° dec. *Anal.* ($C_{11}H_{11}N_3O_3$) C, H, N.

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Isolation and Renin-Inhibitory Activity of Phosphoglyceride from Shark Kidney

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We report on the isolation, fatty acid composition, and renin-inhibitory activity of a chromatographically homogeneous phosphoglyceride from the kidney of the Mako shark, *Isurus oxyrinchus*. Kidney phosphoglyceride from the shark, a lower vertebrate fish, was found to be similar in structure and in activity to mammal (hog) kidney phosphoglyceride; this finding may have some bearing on the question of a possible physiologic role for phosphoglyceride as natural renin inhibitors,[1-5] since renin has been reported[6,7] not to be present in the kidneys of sharks.

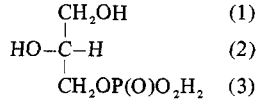
1-Acyl-*sn*-glycerolyso-3-phosphatides† of naturally occurring phosphoglycerides have been reported to inhibit the enzyme renin *in vitro* and the response of injected renin *in vivo*.[2-4] This type of bioactive lysophosphoglyceride, termed *renin inhibitor*,[5] has been shown to be produced *in vitro* by phospholipase A from parent phosphoglyceride, which has been termed *renin preinhibitor*.[5] Parent renin preinhibitor itself has some renin-inhibitory activity *in vitro*[2] but is not nearly as potent as its lysophosphoglyceride; *in vivo*, enzymic hydrolysis of renin preinhibitor to renin inhibitor could be anticipated, but experimental proof of systemic occurrence and distribution of inhibitor-type lysophosphoglyceride is presently lacking. Renin preinhibitor has been isolated from dog and hog kidney[1-3] and also from other organs (heart, spleen, liver) of the rat and dog.[9] Its presence has been demonstrated in plasma and erythrocytes of the rat, dog, and anepheric patients.[9-11] Canine renin preinhibitor reduces plasma reinin activity and blood pressure to near normal levels in both acute and chronic renal hypertensive rats and is reported to have no observed effect on blood pressure of normal rats in spite of causing reduction of plasma renin activity.[4]

Renin preinhibitor from dog and hog kidney is characterized by a high percentage of esterified polyunsaturated fatty acid identified mainly as arachidonate, but the composition and positional distribution of the fatty acids of each individual phosphoglyceride‡ molecule has not been established. Experimental evidence has confirmed that these naturally occurring lipids are phosphatidylethanolamines[5,9,13,14] or related lipids.[5] Identification of renin inhibitor as lysophosphoglyceride also has been indicated by studies[15] with synthetic dilinolenyl phosphatidylethanolamine, which, as the lysophosphoglyceride, was found to have activity nearly comparable to that of natural renin inhibitor and, by inhibition of renin with a number of synthetic lysophosphatidylethanolamines, 2-desoxylysophosphatidylenthanolamines,[13] tridesoxylysophosphatidylethanolamines,[16] and related derivatives.[17]

Isolation. In these studies 1.88 g of crude phosphoglyceride (I) was isolated from 1 kg of Mako shark kidney. Crude I, as analyzed by tlc, consisted of two major and three minor phospholipid components; I contained 3.73% phosphorus and 2.11% nitrogen. Column chromatography of this mixture separated a major homogeneous (tlc) highly unsaturated phosphoglyceride II. The infrared and pmr spectra of II were consistent with those reported[18,19] for naturally occurring phosphoglyceride molecules. The pmr spectrum of II included a broad absorption at δ 8.24–8.68 characteristic of acidic phosphatidylethanolamine polar head protons [-(O)P(O*H*)$OCH_2CH_2NH_2$ and/or -(O)$P(O^-)$-$OCH_2CH_2NH_3^+$].[19] The presence of a CH_2N signal at δ 3.26 further indicated that II is predominantly multispecies phosphatidylethanolamine, since this signal is absent in α-amino acid phosphoglycerides, such as phosphatidylserines; the absence of a signal (δ 3.4)[19] characteristic of trimethylammonium protons revealed that II was not phosphatidylcholine. Chemical shifts of glyceryl, polar head methylene, and saturated and unsaturated fatty acyl chain protons were in accord with literature values;[19] seven distinct absorptions due to protons of esterified polyunsaturated fatty acid were readily assigned when the 100-MHz spectrum of II was compared with spectra of methyl arachidonate, arachidonic acid, and a number of other arachidonyl derivatives.§

The methyl ester compositions of the fatty acids obtained upon methanolysis of II are presented in Table I.

†Stereospecific numbering (*sn*)[8] is used to designate the absolute configuration of naturally occurring phosphoglycerides as derivatives of *sn*-glycero-3-phosphoric acid.



(1) (2) (3)

‡Effective analytical methods are not available for complete separation of intact individual molecules within classes of naturally occurring phosphoglycerides.[12] Therefore, the term renin preinhibitor, when used to describe natural phosphoglyceride isolated from different animal or organ sources, does not necessarily mean phosphoglyceride of identical multispecies but can be considered as homogenous fractions usually indistinguishable by thin-layer chromatography and having similar biological activities.[10]

§J. G. Turcotte, unpublished data.