C^{14}-Labeled Glyceric Acid from G-Gal-Gal-C^{14}.—From 340,000 c.p.m. of G-Gal-Gal isolated in the same chromatograms as the G-Gal above, was obtained 11,000 c.p.m. (9.6% yield) of glyceric acid C^{14}. The time of hydrolysis in hydrochloric acid was 2 hours.

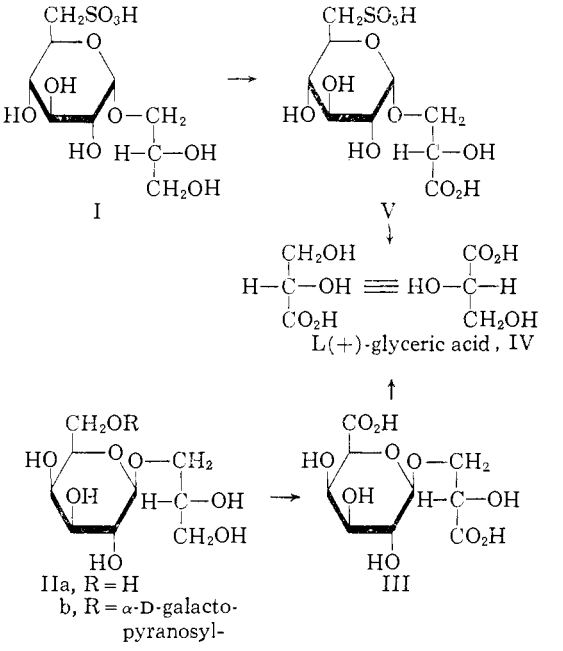
C^{14}-Labeled Glyceric Acid from G-Quin-SO_3H-C^{14}.—G-Quin-SO_3H eluted from the same chromatograms was oxidized as above with nitrogen dioxide, hydrolyzed for 2 hours at 100° with *N* hydrochloric acid. The chromatographically purified glyceric acid was co-crystallized with authentic enantiomorphic salts.

Authentic Crystalline Glyceric Acid Salts.—DL-Glyceric acid was prepared by nitric acid oxidation of glycerol,[11,12] and converted to the quinine salt for resolution.[13] Direct preparation of optically active glyceric acids from the serines[14] was found to be less effective.

Co-crystallization of Glyceric Acids-C^{14} with Authentic Enantiomorphs.—Radioactive glyceric acids were eluted with water from the paper chromatograms to provide standard solutions. Portions (25 μl.) of these were transferred to 25-ml. glass vials in which they were dried and taken up in 75 μl. of 50% ethanol. Dowex-50 (*ca.* 25 mg.) was added to each; the solutions were dried and taken up in 2 ml. of 95% ethanol. Scintillation liquid, 18 ml. (5 g. of PPO and 100 mg. of POPOP per liter of toluene[15]), was added and the samples counted in the Packard Tri-carb[15] liquid scintillation counter. Background counts were made using identical preparations.

Samples of the glyceric acids-C^{14} (*ca.* 100 μl.) containing known amounts of C^{14} were added to *ca.* 20-mg. samples of the authentic crystalline salts in 3-ml. conical centrifuge tubes. The mixture was recrystallized two to five times from hot water with addition of some ethanol when necessary. After removal of mother liquors with a capillary dropper, the salt was dried *in vacuo* and small samples weighed in the counting vials for determination of specific activities. The samples were treated with Dowex-50 in 75 μl. of 50% ethanol and dried. Radioactivity was measured after the addition of 2 ml. of 95% ethanol and 18 ml. of scintillation solution. Results are given in Table I.

To ensure reliability of the method, glycolic acid-C^{14} activity was co-crystallized with D- and L-glyceric acid salts. No radioactivity was retained. Glyceric acid-C^{14}, obtained from chromatograms of photosynthetic products of wheat leaves, co-crystallized quantitatively with D(−)-glyceric quinine salt. The radioactivity was not retained in calcium L(+)-glycerate.



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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY, THE PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNA.]

The Plant Sulfolipid. VII. Synthesis of 6-Sulfo-α-D-quinovopyranosyl-(1→1′)-glycerol and Radiochemical Syntheses of Sulfolipids[1]

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The synthesis of 6-sulfo-O-α-D-quinovopyranosyl-(1→1′)-glycerol and its identity with the sulfoglycosyl glycerol obtained by deacylation of the plant sulfolipid are reported. 6-Sulfo-quinovose was prepared from 1,2-isopropylidene-6-O-tosyl-D-glucofuranose by sulfite replacement. It was converted to the allyl α-glycoside which yielded the desired sulfoquinovosyl glycerol upon oxidation by permanganate. Similarly, S^{35}-labeled sulfoquinovose was converted to the allyl glycoside and to the corresponding dibromide. Radiochemical syntheses of S^{35}-labeled sulfolipids and transglycosidation reactions of 6-sulfo-D-quinovosides-S^{35} are described.

Introduction

The sulfolipid occurring in photosynthetic tissues was presumed to be a sulfoglycosyl glyceride[4] on the basis of radiochromatographic evidence. Mild saponification of the sulfolipid yielded a sulfoglycosylglycerol which was isolated as a crystalline salt after purification by anion exchange resin chromatography.[5] The sulfosugar liberated by acid hydrolysis of the sulfoglycosylglycerol was recently identified[6] as 6-sulfo-D-quinovose (6-deoxy-D-glucose-6-sulfonic acid) (I). The α-glucosidic linkage in the natural glycoside was also demonstrated.

In this paper are described syntheses of 6-sulfo-D-quinovose,[6a] 6-sulfo-O-α-D-quinovopyranosyl-(1 → 1)-glycerol and S^{35}-labeled sulfolipid which are the first carbohydrates containing the sulfonic acid group to be recognized in Nature.

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Results and Discussion

1,2-O-Isopropylidene-6-O-tosyl-D-glucofuranose[7] underwent a sulfite replacement reaction with sodium sulfite to afford 1,2-O-isopropylidene-6-sulfo-D-quinovose. Hydrolysis resulted in production of 6-sulfo-D-quinovose (I) isolated as the barium salt in an over-all yield of 66%. The sulfosugar was converted into methyl 6-sulfo-α-D-quinovopyranoside (IIa) which was isolated as the crystalline cyclohexylamine salt and was identical with the methyl sulfoglycoside prepared from the sulfosugar of natural origin.[8] The α-glucosidic linkage in IIa was clear from its molecular rotation, $[M]^{25}D$ + 31,000°, and the rotatory dispersion curve. The crystalline cyclohexylamine salt of allyl 6-sulfo-α-D-quinovopyranoside (IIb) was obtained from I by treatment with boiling allyl alcohol followed by neutralization with cyclohexylamine. No catalyst was used in either case, since I bears the strongly acidic sulfonic acid group. The fact that no β-isomer was isolated is not unusual.[9] Permanganate oxidation of IIb gave, after purification by ion exchange chromatography, 6-sulfo-O-α-D-quinovopyranosyl-(1→1)-DL-glycerol (IIc) as a thick sirup (cyclohexylamine salt) which was indistinguishable from the natural sample in its chromatographic behavior on paper as well as on anion exchange resin columns. Nearly half of the synthetic product was isolated from the sirup as fine crystals (from methanol–toluene) which showed, after recrystallization from the same solvent, the same melting point, mixed melting point, infrared[10] and nuclear magnetic resonance spectra[10] as the natural sulfoglycosylglycerol (cyclohexylamine salt). Since the latter was demonstrated to possess the D-glycerol structure,[11] the crystalline synthetic product must be also the D-glycerol isomer. The permanganate oxidation yields both D- and L-isomers; therefore, the non-crystalline portion must contain the L-glycerol isomer. The identity of the synthetic crystalline D-compound with S^{35}-labeled sulfoglycosylglycerol[4] isolated from *Chlorella pyrenoidosa* was confirmed by quantitative retention of the radioactivity after repeated corecrystallization.

The total radiochemical synthesis of sulfolipids was effected in three ways. The starting material, allyl 6-sulfo-D-quinovoside-S^{35}, was prepared by heating 6-sulfo-D-quinovose-S^{35} in allyl alcohol in the presence of Dowex-50 followed by separation on paper. The product was presumed to be largely the α-D-pyranoside (IIb) by analogy with the unlabeled compound; IIb-S^{35} gave 6-sulfo-D-quinovopyranosyl-(1→1′)-DL-2′,3′-dibromopropanol (IIb) by bromination. It was subjected to acyl replacement with silver or potassium palmitate in the presence of trace amounts of sodium iodide. Chromatographic separation revealed formation in good yield of a sulfolipid which was presumed to be IIe from R_f values and also from the following hydrolysis experiments. Mild alkaline hydrolysis of IIe gave a single spot which was presumed to be 6-sulfo-O-α-D-quinovopyranosyl-(1→1′)-2′-bromo-3′-hydroxy-propanol (IIf). Acid hydrolysis of IIf gave a single spot which was identical with 6-sulfo-D-quinovose (I). Another spot was also found in the synthetic mixture which had slightly higher R_f values than IIe and exactly the same coördinates as the natural sulfolipid IIg; however, its yield was less than 2.0%. The second synthesis was an application of the Prévost reaction; IIb-S^{35} was heated with iodine and silver palmitate in benzene or dioxane. Chromatographic separation showed radioactivity at the position corresponding to IIg; however, the yield was less than a few per cent. The third synthesis was performed by transglycosidations between IIb-S^{35} and glycerides. Taking into account the concurrent transacylation reactions the product contained some isomers with reference to the position of acyl groups. In addition, a transglycosidation between IIb-S^{35} and glycerol monoöleate afforded 6-sulfo-O-D-quinovopyranosyl-(1 → 1′)-3′-O-oleoyl-glycerol-S^{35} (IIh) and 6-sulfo-O-D-quinovofuranosyl-(1 → 1′)-3′-O-oleoylglycerol-S^{35} (IIIc). The structures IIh and IIIc were deduced from results of enzymatic degradation. Other transglycosidation reactions of the sulfosugar were also carried out and the results are summarized in Table I.

Experimental Part

6-Sulfo-D-quinovose (6-Deoxy-D-glucose-6-sulfonic Acid). To a solution of 20 g. of 1,2-O-isopropylidene-6-O-tosyl-D-glucofuranose[7] in 200 ml. of ethanol was added 12 g. of sodium sulfite[12] dissolved in 200 ml. of water and the mixture was boiled 24 hours under reflux, 200 ml. of water was added and the solution concentrated to 250 ml. *in vacuo*, freed from sodium ion by passing through Dowex 50 and further concentrated *in vacuo*, below 40°, to a thick sirup. The remaining sulfur dioxide was removed by further addition of water followed by concentration. The isopropylidene group hydrolyzed spontaneously during this operation. The sirup containing an equimolecular amount of 6-sulfo-D-quinovose and *p*-toluenesulfonic acid was neutralized with barium carbonate and barium hydroxide to *p*H 7.0. The neutral solution was clarified by filtration and concentrated to a crystalline mass which was boiled with 1 l. of 95% ethanol. The alcohol dissolved the crystals, leaving a paste remaining at the bottom of the flask, which was dried over P_2O_5 *in vacuo*. The yield was 11.0 g. (66%).

Anal. Calcd. for $C_6H_{11}O_8S$ Ba/2: Ba, 22.02. Found: Ba, 21.13.

Additional crops were obtained from the ethanolic decant by concentration, filtration of crystalline barium *p*-toluenesulfonate, concentration of the filtrate and treatment with hot ethanol.

6-Sulfo-D-quinovose obtained above as the barium salt showed, after being freed from barium, a single spot upon paper chromatography in several different solvent systems. It gave a positive reaction to the aniline–trichloroacetic acid spray reagent, to the periodate–SO_2–fuchsin reagent and also to brom thymol blue. The R_f values were identical with those of the hexosesulfonic acid of natural origin. The synthetic sulfosugar contained less than 1% barium *p*-toluenesulfonate as estimated from its ultraviolet absorption. The correctness of the structure was demonstrated by conversion to alkyl glycosides.

Methyl 6-Sulfo-α-D-quinovopyranoside Cyclohexylamine Salt.—Three grams of the barium salt of 6-sulfo-D-quinovose dissolved in 50 ml. of water was passed through a Dowex 50 column. The combined effluate and washings were concentrated *in vacuo* to a thick sirup at below 40°

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TABLE I

REACTION CONDITIONS AND PRODUCTS

Starting material	Reagent	Conditions: Temp., °C.	Conditions: Time, hr.	Main product	Yield, %	Minor product	Yield, %
I	Allyl alcohol, Dowex 50	100	10 in sealed tube	IIb	50.5	Many	..
I	2,3-Dibromopropanol, Dowex 50	100	12 in sealed tube	IId	58.0	IIIa	17.4
I	1,3-Dibromoisopropyl alc., Dowex 50	100	20 in sealed tube	IIIb	*ca.* 20	IIi	*ca.* 5
IIb	Bromine		5 min. room temp. in EtOH–CCl_4	IId	52.6	None	..
IIc	Allyl alcohol, Dowex 50	100	10 in sealed tube	IIb	50.9	Many	..
IIg	Allyl alcohol, Dowex 50	100	10 in sealed tube	IIb	55.6	Many	..
IIc	1,3′-Dibromoisopropyl alc., Dowex 50	100	12 in sealed tube	IIIb	11.6	IIi	6
IIc	2,3-Dibromopropanol, Dowex 50	100	12 in sealed tube	IId	34.1	IIIa	19
IIg	2,3-Dibromopropanol, Dowex 50	100	11 in sealed tube	IId	43.2	IIIa	19
IId	Silver palmitate, trace of NaI	100	40 in acetonitrile, sealed tube	IIe	8.0	IIg	0.5
IId	Potassium palmitate, NaI	100	40 in acetonitrile, sealed tube	IIe	5.0	IIg	0.2
IId	Silver palmitate, trace of NaI	135	13 in dimethylformamide, sealed tube	IIe	>50	IIg	2.0
IId	Potassium palmitate, NaI	135	13 in dimethylformamide, sealed tube	IIe	>40	IIg	0.9
IIb	Silver palmitate, iodine	100	48 in benzene, sealed tube	IIg	2–3	Many	..
IIb	Silver palmitate, iodine	100	48 in dioxane, sealed tube	IIg	2	Many	..
IIb	Glycerol monoöleate	100	18 in dioxane, sealed tube	IIIc[a]	27	IIh[a]	10
IIe	0.1 *N* KOH in methanol		Overnight at room temp.	IIf	80	None	
IIf	2 *N* HCl	100	2	I	70	None	

[a] Sulfolipid produced was hydrolyzed by a specific sulfolipase of *Scenedesmus* prepared by T. Yagi.

TABLE II

R_f VALUES OF SULFOSUGAR DERIVATIVES[a]

Compound	PW[b]	BPAW[c]	Compound	PW[b]	BPAW[c]
IIb	0.39	0.32	IIIb	0.54	0.36
IId	.42	.39	IIe	.67	.66
IIIa	.54	.39	IIf	.42	.20
IIi	.39	.35	IIg	.67	.70

[a] Whatman No. 4 paper, 20°. [b] Phenol–water solvent. [c] Butanol–propionic acid–water solvent.

(bath temp.) and dried over P_2O_5 in high vacuum at room temperature to a foamy glass. This was dissolved in 150 ml. of methanol, refluxed 72 hours under exclusion of atmospheric moisture, cooled, neutralized to *p*H 6 with cyclohexylamine, and concentrated *in vacuo*. The final gum was dissolved in a small amount of ethanol and crystallized by careful addition of ethyl acetate. The crude crystals were collected, washed with ethanol–ethyl acetate (1:1) then with ethyl acetate (yield 1.13 g.). After recrystallization from ethanol–toluene it melted at 173–174°, $[M]^{25}D$ +31,133° (*c* 4.16, water), $[M]^{25}Hg_{365}$ +91,210° (*c* 4.16, water).[13] Infrared[10] and nuclear magnetic resonance spectra[10] were consistent with the anticipated structure.

Anal. Calcd. for $C_{13}H_{27}O_8NS$: C, 43.68; H, 7.61; N, 3.92; S, 8.97. Found: C, 43.47; H, 7.43; N, 3.84; S, 8.94.

Allyl 6-Sulfo-α-D-quinovoside Cyclohexylamine Salt.—Forty grams of the barium salt of 6-sulfo-D-quinovose was dissolved in water, freed from barium by passing through a Dowex 50 column, concentrated to a thick sirup at below 40° and dried over phosphorus pentoxide *in vacuo*. The product was dissolved in 50 ml. of anhydrous allyl alcohol, evaporated to dryness, dissolved again in 50 ml. of allyl alcohol and heated on a steam-bath for 12 hours. The reaction mixture was cooled, neutralized with cyclohexylamine to *p*H 6.5–7.0, concentrated *in vacuo* and dissolved in 50 ml. of ethanol–ethyl acetate (15:85). The crystals, 19.0 g., were collected by suction, washed with ethanol–ethyl acetate and dried. The filtrate was concentrated, dissolved in water, treated while hot with active carbon, freed from cyclohexylamine by passing through Dowex 50, concentrated and dried *in vacuo*. An additional 8.0 g. of the desired product was obtained by allyl alcohol treatment. The total yield was 27.0 g. (55.0%). After recrystallization from ethanol–ethyl acetate it melted at 151.5–153°, $[M]^{25}Na_{589}$ +32,967° (*c* 4.22, water), $[M]^{25}Hg_{365}$ +96,566° (*c* 4.22, water).[12]

(13) Measured by Dr. M. Lepage.

Anal. Calcd. for $C_{15}H_{29}O_8NS$: C, 46.99; H, 7.62; N, 3.65; S, 8.36. Found: C, 46.42; H, 7.55; N, 3.62; S, 8.43.

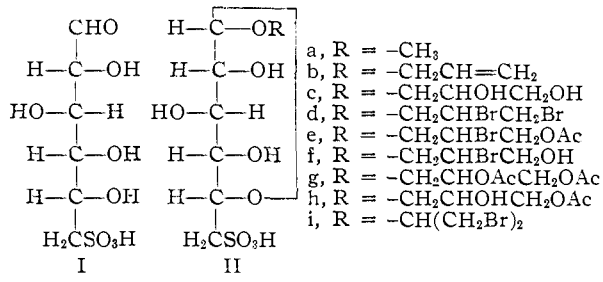
6-Sulfo-O-α-D-quinopyranosyl-(1→1)-glycerol Cyclohexylamine Salts.—The cyclohexylamine salt (2.24 g.) of allyl 6-sulfo-α-D-quinovoside was dissolved in water and freed from cyclohexylamine by passing through a Dowex 50 column (1 × 18 cm.). The eluate (150 ml.) in which 2.0 g. of potassium hydroxide (or potassium carbonate) and 150 ml. of ethanol was dissolved was treated with 1.2 g. of potassium permanganate[14] in a minimum volume of water during 1 hour with vigorous shaking and cooling in an ice-bath. It was shaken for additional 5 hours at room temperature. The manganese dioxide was removed by suction filtration through Hyflo–Super-Cel. The filtrate was concentrated to half its volume and passed through a Dowex 50 column (3 × 18 cm.) to remove potassium ions. The filtrate and the washings were combined and concentrated *in vacuo*. This was repeated five times to remove acetic acid. The product was absorbed on a Dowex 1-X8 (formate form) column (4.3 × 13 cm.) and eluted with formic acid in aqueous ammonium formate (gradient concentration). The eluate (750 ml.) was collected as a main fraction which was easily recognized by brown coloration when an aliquot was evaporated under an infrared lamp.[5] Ammonium ion was removed by passing through a Dowex 50 column (3 × 21 cm.). Concentration of the filtrate *in vacuo* was repeated more than five times after addition of water to remove formic acid completely. The concentrate was neutralized with cyclohexylamine and evaporated to dryness (80° in high vacuum over P_2O_5). The resulting almost colorless glass amounted to 1.17 g. which was certainly a mixture of stereoisomers with reference to the C-2 of glycerol. Paper chromatograms of this glass showed the same spot as sulfoglycosylglycerol-S^{35} of natural origin. The glass was dissolved in warm ethanol and crystallization was effected by careful addition of toluene. The crystals were collected, washed with 95% ethanol–toluene (3:7) and recrystallized from methanol–toluene; yield 0.42 g., m.p. 191–193°, mixed melting point with a natural sample showed no depression.

Anal. Calcd. for $C_{15}H_{31}O_{10}NS$ (417.5): C, 43.15; H, 7.49; N, 3.36; S, 7.68. Found: C, 43.35; H, 7.62; N, 3.03, 3.67; S, 7.94.

An additional crop was obtained from the mother liquors.

Infrared and nuclear magnetic resonance spectra[10] were indistinguishable from those of the sulfoglycosyl glycerol of natural origin.

(14) Amounts of potassium permanganate ranging from 0.96 to 1.46 g. were used but the results were almost the same.



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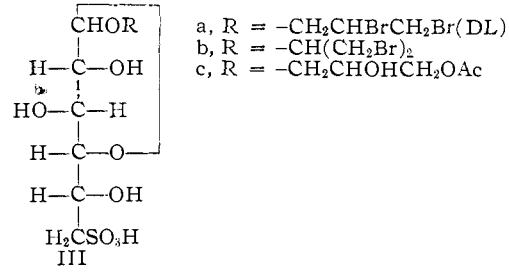

III

Co-recrystallization of Natural and Synthetic Sulfoglycosylglycerols.—A spot of sulfoglycosylglycerol-S^{35} in a radiogram prepared from the natural sulfolipid-S^{35} was cut out and eluted with water to make the standard solution. A portion (25 μl.) of the standard solution was transferred to a 25-ml. bottle for scintillation counting, dried and dissolved in 2 ml. of 95% ethanol. 18 ml. of scintillation liquid (5 g. of PPO and 100 mg. of POPOP in 1 l. of pure toluene) was added and the radioactivity was counted in the Packard Tricarb scintillation counter (Packard Inst. Co., LaGrange, Ill.).

Co-crystallization was carried out using 75 μl. (32,700 c.p.m.) of the standard solution and 16.7 mg. of the synthetic 6-sulfo-O-α-D-quinovopyranosyl(1→1)-glycerol cyclohexylamine salt. The mixture was dried and recrystallized from methanol–toluene. The specific radioactivity after three recrystallizations was 1,980 c.p.m./mg. After five recrystallizations the specific activity was found to be 1,910 c.p.m./mg., indicating quantitative retention of the S^{35}.

Acknowledgment.—We would like to express our appreciation to Dr. R. O. Mumma for his collaboration in obtaining and interpreting the infrared and nuclear magnetic resonance spectra, to Dr. M. Lepage for optical rotation measurements and to Dr. I. Shibuya and Mr. T. Yagi for their helpful suggestions.

[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS]

Proton Magnetic Resonance of Nucleotides. IV. Ribose Conformation[1,2]

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Analysis of proton magnetic resonance spectra of the cyclic and monocyclic monocleotide isomers leads to the assignment of specific conformations for the sugar ring. In particular, the spacings in the multiplets due to H_1', H_2' and H_3' agree with a C_2'-*endo* conformation for 2′-AMP in which C_2' is displaced by about 0.5 Å. relative to the ring plane and C_4' is also slightly rotated in the opposite direction. This analysis is valid only if the theory relating coupling constants and dihedral angles in these compounds is quantitatively correct. Increased confidence in defining relative proton orientation from coupling constants in these compounds is based on the excellent agreement between the observed and calculated spin coupling constant, $J_{1'-2'}$, in the case of the cyclic 3′:5′-AMP whose sugar conformation (C_3'-*endo* — C_4'-*exo*) is determined solely by steric requirements.

Specific conformations for the D-ribofuranose ring of purine and pyrimidine nucleosides have been previously suggested on the basis of the magnitude of the spin coupling constant between the protons on adjacent carbon atoms of the sugar ring.[3] Conformations for thymidine[4,5] and for other deoxyribonucleosides and nucleotides[6] have also been suggested.

The dependence of the spin coupling constant on the angle formed by the two intersecting planes defined by H_1–C–C and C–C–H_2 in compounds of the type H_1–C–C–H_2 (dihedral angle, ϕ) has been shown theoretically to be described by the function $8.5\cos^2\phi - 0.28$ (eq. 1) for angles from 0 to 90° and $9.5\cos^2\phi - 0.28$ (eq. 2) for angles from 90 to 180°.[7] Conroy[8] has published a slightly different function for angles from 90 to 180°. The experimentally determined coupling constants from p.m.r. studies on small molecules (substituted ethanes, cyclohexanes, the acetylated aldopyranoses and camphane-2:3-diols) are in good agreement with Karplus' theoretically predicted values.[9,10]

In order to establish the principles governing sugar conformation in the nucleic acid derivatives, a p.m.r. study was made of the various adenylic acid isomers and of the cyclic phosphates of cytidine and uridine. If the magnitude of the coupling constant indeed reflects the relative orientation of the protons on the first and second carbon atoms of D-ribose (and hence the pucker of the 5-membered ring) one would expect that it might vary depending on the position of the phosphate group on the ring.

The spectra and chemical shifts for the protons of the various nucleotides are seen in Fig. 1 The experimentally determined coupling constants be-

(1) This investigation was supported by a Special Research Fellowship from the Public Health Service, and by grants to Professor J. T. Edsall from the Public Health Service (H-3169) and from the National Science Foundation (G-9116).

(2) Abbreviations used: 2′-AMP, 3′-AMP and 5′-AMP = 2′-, 3′-, and 5′-adenosine monophosphate; 2′:3′-AMP and 3′:5′-AMP = 2′:3′-cyclic and 3′:5′-cyclic adenosine monophosphate; 2′:3′-CMP = 2′:3′-cyclic cytidine monophosphate and 2′:3′-UMP = 2′:3′-cyclic uridine monophosphate.

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