Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Phospholipids in Solution. Spectral and Stereochemical Assignments Based on $^{13}C-^{31}P$ and $^{13}C-^{14}N$ Couplings[1]

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Natural-abundance carbon-13 NMR spectra of various synthetic and natural phospholipids of biochemical interest were measured in chloroform–methanol–water (50:50:15, by volume). In this solvent, nonaggregation is indicated by narrow resonances, by reproducible chemical shifts, and by the consistency of two-bond and three-bond carbon–phosphorus couplings which were observed for phospholipids containing a choline, ethanolamine, or bromoethanol headgroup. The choline phospholipids also showed well-defined $^{13}C-^{14}N$ couplings. Further substitution of alkyl for acyl side chains or of diols for the glycerol backbone permitted unequivocal assignment of headgroup and backbone carbon resonances. The magnitude of $^{3}J_{CP}$ couplings (averaging 7.6 Hz) clearly demonstrates that the respective carbons (C_B) of the headgroup and backbone (C_β) of a wide range of choline and ethanolamine phospholipids in solution prefer trans orientation relative to the phosphorus; only in 1,3-diacylglycero-2-phosphocholine, the glycerol backbone remains essentially in gauche conformation. Observation of carbon–phosphorus and carbon–nitrogen couplings and $^{2}J_{CP}$ and $^{3}J_{CP}$ values are shown to be useful criteria in assigning chemical shifts in ^{13}C NMR spectra of phospholipids.

Carbon-13 nuclear magnetic resonance (NMR) spectroscopy[2] is often the method of choice for defining structural parameters, conformations, and interactions of complex biomolecules in solution. Yet, most phospholipids, due to their amphiphilic nature, resist solubilization in polar as well as nonpolar solvents by forming liposomes or reverse micelles, respectively.

We have recently shown[3] that $^{13}C-^{14}N$ couplings (J_{CN}) and ^{14}N quadrupolar relaxations (T_1) of choline phospholipids in various media are sensitive to changes in the phospholipid polar headgroup region. We also found that observance of $^{13}C-^{14}N$ couplings and the magnitude of ^{14}N T_1 are not dependent upon phospholipid–solvent interactions, but rather are indicators of aggregation or nonaggregation of the phospholipid molecules in a given medium. On the basis of these criteria and on results presented here, chloroform–methanol–water (50:50:15, by volume) was chosen as solvent in order to minimize molecular interactions between phospholipid species, even at relatively high concentrations (~100 mg/mL).

In the present study, natural-abundance carbon-13 NMR spectra of a series of synthetic and natural phospholipids containing a choline, ethanolamine, or bromoethanol headgroup were measured in solution. Chemical shift assignments were made possible by following the effect of substitution after modifying the long-chain acyl and alkyl moieties and the glycerol and diol backbones, as well as the polar headgroup functions. The study also showed that observation of well-resolved $^{13}C-^{31}P$ couplings (J_{CP}) does, in fact, aid in the assignment of the phospholipid backbone and headgroup carbon resonances. Three-bond carbon–phosphorus couplings ($^{3}J_{CP}$) were found particularly sensitive to conformational changes of the phospholipid molecules in the solvated state.

Experimental Section

Carbon-13 NMR spectra were recorded at 20 MHz on Varian CFT-20 and FT-80A pulse Fourier-transform instruments. Spectra were measured with 30–100 mg/mL samples (10-mm o.d. sample tubes) at the ambient probe temperature of 37 ± 1 °C. $CDCl_3$–CD_3OD–D_2O (50:50:15, by volume)[3] served as solvent and for field frequency locking purposes. ^{13}C NMR spectra were obtained with broad band proton decoupling, using 8K data points for a spectral width of 4000 Hz. Chemical shifts are expressed in parts per million downfield from Me_4Si, which served as internal standard.

The phospholipids used in this study were synthesized[4,5] or isolated[6,7] in our laboratory (for details, see Table I). The compounds were pure as judged by thin-layer chromatography (silica gel H, Merck); developing solvent was chloroform–methanol–water, (65:35:8, by volume). Fractions were made visible by charring.

Results and Discussion

The proton-decoupled carbon-13 NMR spectra of various phospholipids bearing a choline (**1–15**), ethanolamine (**16–21**), or bromoethanol (**22–26**) headgroup and containing an alkyl (**1, 16**), ethanediol (**2–6, 17, 22, 23**), 1,3-propanediol (**7, 8, 18, 24, 25**), or glycerol (**9–15, 19–21, 26**) backbone were measured, using deuterated chloroform–methanol–water (50:50:15) as solvent.[3] In this medium, the phospholipids **1–26** were readily soluble at concentrations that permitted rapid recording of natural-abundance carbon-13 spectra. The spectra showed consistent chemical shifts for the carbons of the respective phospholipid backbone, base, and lipophilic moieties and were characterized by well-resolved resonances and well-defined carbon–phosphorus and carbon–nitrogen couplings. The 20-MHz ^{13}C spectrum of 1-*O*-hexadecyl-2-*O*-[[2-(trimethylamino)ethyl]phosphoryl]ethanediol (**2**) is given as a typical example (Figure 1). The insert of Figure 1

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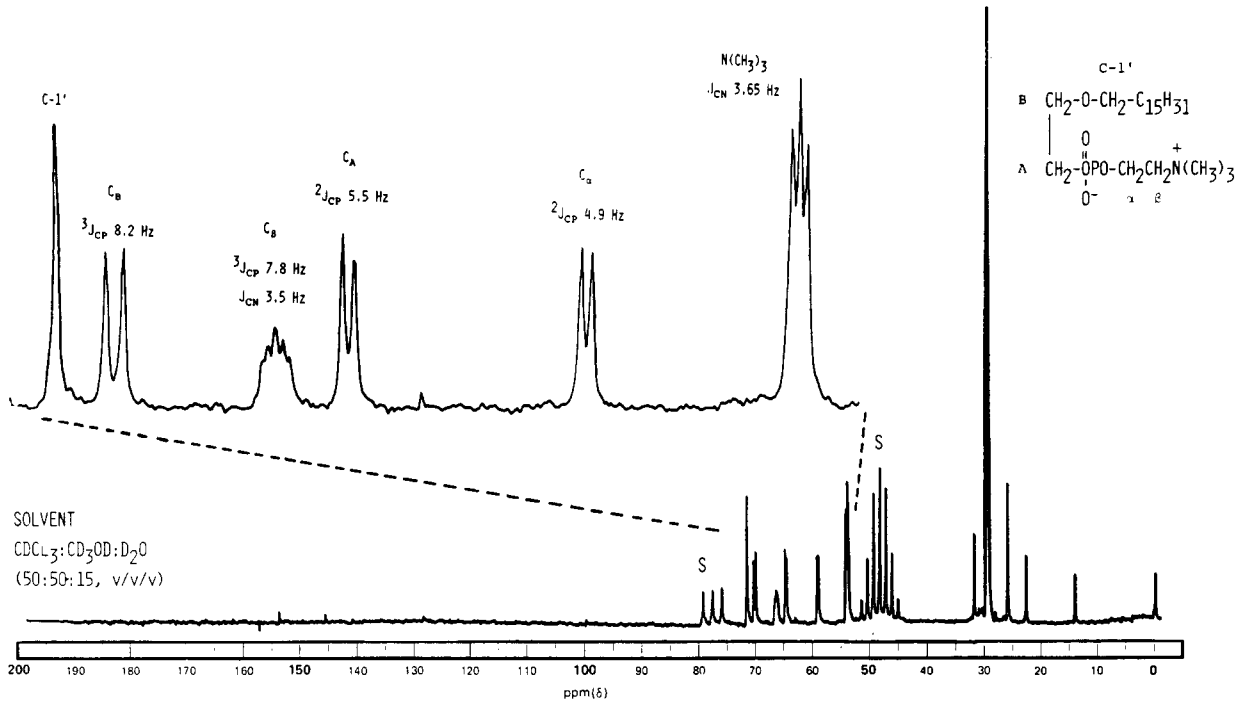

Figure 1. 20-MHz ^{13}C NMR spectrum of 1-*O*-hexadecyl-2-*O*-[[2-(trimethylamino)ethyl]phosphoryl]ethanediol (**2**) in $CDCl_3$–CD_3OD–D_2O (50:50:15, by volume). The insert shows the resonances of alkyl CH_2O (71.89, s, C-1′), glycol CH_2OR (70.52, d, $^3J_{CP}$ = 8.2 Hz, C_B), CH_2N (66.76, dt, $^3J_{CP}$ 7.8 Hz, J_{CN} = 3.5 Hz, C_β), glycol CH_2OP (65.04, *d*, $^2J_{CP}$ = 5.5 Hz, C_A), choline CH_2OP (59.49 *d*, $^2J_{CP}$ = 4.9 Hz, C_α), and $N(CH_3)_3$ (54.42, t, J_{CN} = 3.65 Hz).

illustrates the types of line shapes and splittings that were observed. The chemical shift, ^{13}C–^{14}N couplings, and the two-bond and three-bond ^{13}C–^{31}P couplings of the phospholipids **1–26** used in the present study are compiled in Table I.

Chemical Shift Analysis Based on Substitution Effect. The assignment of the headgroup and backbone carbon resonances of phospholipids **1–26** was possible by following the effect of substitution at specific phospholipid sites on the carbon chemical shifts. Within the series of choline phospholipids (**1–15**), ethanolamine phospholipids (**16–21**), or bromoethanol intermediates (**22–26**), changes in the polyol or aliphatic functions did not affect the chemical shifts of the polar headgroup carbons C_α and C_β, or of $N(CH_3)_3$ (Table I). Independent of the type of lipophilic backbone, the headgroup carbon resonances consistently appeared at 59.58 ± 0.15 (C_α), 66.80 ± 0.15 (C_β), and 54.47 ± 0.05 ppm ($N(CH_3)_3$) for the choline phospholipids **1–14**, at 62.16 ± 0.14 (C_α) and 40.80 ± 0.07 ppm (C_β) for the ethanolamine phospholipids **16–21**, and at 66.10 ± 0.33 (C_α) and 31.27 ± 0.13 ppm (C_β) for the bromoethanol intermediates **22–26**. The signals of C_α and C_β, in turn, were readily distinguished by following the effect of changes in substitution at the C_β methylene group. Thus, exchange of $N(CH_3)_3$ for NH_2 or Br caused the upfield shift of C_β from 66.8 to 40.8 or 31.3 ppm, respectively, while C_α responded to a much lesser extent to this type of substitution (59.6 vs. 62.2 vs. 66.1 ppm). A direct comparison of the C_α and C_β chemical shifts of the 1-*O*-hexadecyl-1,3-propanediol (**7**, **18**, **24**) or 1,2-di-*O*-hexadecanoylglycerol (**10**, **19**, **26**) derived choline (**7**, **10**), ethanolamine (**18**, **19**), and bromoethanol (**24**, **26**) phospholipids illustrates this phenomenon particularly well.

As changes in the backbone structural features did not affect the chemical shift of the headgroup carbons, changes in the headgroup did not cause significant shifts for the backbone carbons C_A, C_B, and C_C or for the carbons of the aliphatic chains. Thus, the signals of C_A (67.21 ± 0.01), C_B (69.10 ± 0.02), and C_C (65.55 ± 0.01) of the choline (**14**) and ethanolamine (**21**) lysophospholipids were identical, and the same holds true for C_A and C_B of the long-chain alkyl phosphocholine **1** and phosphoethanolamine **16**, for example, or for the alkyl ethanediol ether phospholipids **2**, **17**, and **22**.

Alkyl vs. acyl substitution, as expected, affected foremost the backbone carbon bearing the substituent and to a lesser extent adjacent backbone carbons. This is illustrated well by comparing the C_B shifts of the ethanediol ether phosphocholines **2** and **3** occurring at 70.54 ± 0.02 ppm with those of the respective esters **4–6** at 64.24 ± 0.02 ppm. Similar trends are apparent for the 1,3-propanediol ether **7** vs. the 1,3-propanediol ester **8**, or for the glycerol diether **9** vs. the glycerol diesters **10**, **11**, and **13**, as well as for other ether/ester pairs (Table I).

The backbone carbon C_A adjacent to the phosphate function is readily distinguished from the headgroup carbon C_α on the basis of the observations that (i) the chemical shift of C_α is affected to some extent by changes in substitution at C_β (e.g., **10** vs. **19** vs. **26** as shown above), whereas C_A is not, and (ii) that the chemical shift of C_A varies with changes in substitution at the adjacent carbon C_B (e.g., **2** and **3** vs. **4**, **5**, and **6**), while such structural modification does not at all affect C_α.

Assignments of the aliphatic carbons of the saturated and unsaturated side chains (see Table I) are consistent with data reported in the literature.[12] However, disagreement exists in the literature[13–15] as to the assignment of the ^{13}C NMR signals of the glycerol backbone and choline headgroup carbons of phosphatidylcholines. Our data confirm the early assignments made by Birdsall et

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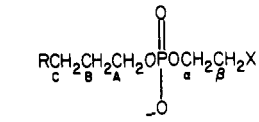
Table I. ^{13}C NMR Chemical Shifts and ^{13}C–^{31}P and ^{13}C–^{14}N Couplings of Various Phospholipids in Solution[a]

phospholipid	no.	backbone and headgroup carbon assignments					
		C_A[b]	C_B[c]	C_C	C_α[b]	C_β[d]	$N(CH_3)_3$[e]
hexadecyl 2-(trimethylamino)ethyl phosphate	1[f]	66.50 (5.8)	31.10 (7.5)		59.43 (4.7)	66.66[g]	54.46
1-*O*-hexadecyl-2-*O*-[[2-(trimethylamino)ethyl]phosphoryl]ethanediol	2[h]	65.04 (5.5)	70.52 (8.2)		59.49 (4.9)	66.76 (7.8)	54.42
1-*O*-(hexadec-*cis*-9-enyl)-2-*O*-[[2-(trimethylamino)ethyl]phosphoryl]ethanediol	3[h]	65.09 (5.3)	70.56 (8.0)		59.54 (4.8)	66.75 (7.6)	54.46
1-*O*-hexadecanoyl-2-*O*-[[2-(trimethylamino)ethyl]phosphoryl]ethanediol	4[f]	63.95 (4.9)	64.26 (7.3)		59.73 (4.7)	66.83 (7.2)	54.50
1-*O*-(hexadec-*cis*-9-enoyl)-2-*O*-[[2-(trimethylamino)ethyl]phosphoryl]ethanediol	5[h]	63.89 (5.5)	64.23 (8.2)		59.59 (5.2)	66.74 (7.2)	54.45
1-*O*-(octadec-*cis*-9-enoyl)-2-*O*-[[2-(trimethylamino)ethyl]phosphoryl]ethanediol	6[h]	63.89 (5.5)	64.22 (8.2)		59.60 (4.9)	66.77 (7.5)	54.46
1-*O*-hexadecyl-3-*O*-[[2-(trimethylamino)ethyl]phosphoryl]propanediol	7[h]	63.44 (5.6)	31.11 (7.3)	67.72	59.46 (4.9)	66.91 (7.5)	54.47
1-*O*-hexadecanoyl-3-*O*-[[2-(trimethylamino)ethyl]phosphoryl]propanediol	8[h]	62.85 (5.5)	30.19[g]	61.87	59.55 (4.8)	66.82 (7.9)	54.47
1,2-di-*O*-hexadecyl-3-*O*-[[2-(trimethylamino)ethyl]phosphoryl]-*rac*-glycerol	9[i]	65.54 (5.4)	78.95 (8.3)	72.16	59.61 (4.2)	66.89 (7.3)	54.48
1,2-di-*O*-hexadecanoyl-3-*O*-[[2-(trimethylamino)ethyl]phosphoryl]-*rac*-glycerol	10[f]	64.08 (4.5)	70.97 (7.7)	63.30	59.71 (3.9)	66.85 (7.2)	54.52
1,2-di-*O*-(octadec-*cis*-9-enoyl)-3-*O*-[[2-(trimethylamino)ethyl]phosphoryl]-*rac*-glycerol	11[j]	64.00 (4.8)	70.92 (7.9)	63.23	59.62 (4.8)	66.71 (7.6)	54.44
1,3-di-*O*-hexadecanoyl-2-*O*-[[2-(trimethylamino)ethyl]phosphoryl]-*rac*-glycerol	12[f]	71.20 (4.9)	63.31 (3.9)		59.72 (4.6)	66.95 (7.9)	54.50
1,2-di-*O*-acyl-*sn*-glycero-3-phosphocholine (egg yolk PC)	13[k]	64.02 (4.8)	70.94 (7.9)	63.26	59.65 (4.8)	66.79 (7.0)	54.45
1-*O*-acyl-*sn*-glycero-3-phosphocholine (from egg yolk PC)	14[l]	67.22 (5.6)	69.08 (7.2)	65.54	59.71 (4.8)	66.67[g]	54.47
sn-glycero-3-phosphocholine	15[m]	68.13 (5.9)	71.10 (7.7)	62.65	60.64 (4.9)	66.54 (7.7)	54.54
octadecyl 2-aminoethyl phosphate	16[n]	66.61 (5.0)	31.07 (6.9)		62.07 (br)	40.83 (br)	
1-*O*-hexadecyl-2-*O*-[(2-aminoethyl)phosphoryl]ethanediol	17[n]	65.13 (5.4)	70.56 (8.1)		62.19 (5.1)	40.87[c] (6.5)	
1-*O*-hexadecyl-3-*O*-[(2-aminoethyl)phosphoryl]propanediol	18[n]	63.53 (5.6)	31.07 (7.2)	67.69	62.02 (5.3)	40.83[c] (6.3)	
1,2-di-*O*-hexadecanoyl-3-*O*-[(2-aminoethyl)phosphoryl]-*rac*-glycerol	19[n]	64.11 (4.6)	70.93 (8.2)	63.24	62.13 (4.7)	40.86[c] (6.0)	
1,2-di-*O*-acyl-*sn*-glycero-3-phosphoethanolamine (egg yolk PE)	20[k]	64.07 (4.7)	70.88 (7.5)	63.16	62.07 (4.8)	40.74[c] (6.2)	
1-*O*-acyl-*sn*-glycero-3-phosphoethanolamine (from egg yolk PE)	21[l]	67.21 (5.5)	69.11 (7.6)	65.56	62.29 (4.6)	40.86[c] (7.0)	
1-*O*-hexadecyl-2-*O*-[(2-bromoethyl)phosphoryl]ethanediol	22[h]	65.16 (3.5)	70.47 (8.5)		65.77 (3.3)	31.29[c] (7.5)	

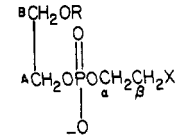
1-*O*-(octadec-*cis*-9-enoyl)-2-*O*-[(2-bromoethyl)phosphoryl]ethanediol	**23**[h]	63.98 (5.0)	64.30 (8.1)		65.79 (5.4)	31.14[c] (7.7)
1-*O*-hexadecyl-3-*O*-[(2-bromoethyl)phosphoryl]propanediol	**24**[h]	64.50 (5.7)	29.94[g]	67.24	66.42 (4.9)	31.28[c] (7.9)
1-*O*-hexadecanoyl-3-*O*-[(2-bromoethyl)phosphoryl]propanediol	**25**[h]	62.75 (4.9)	29.97[g]	62.23	65.78 (4.5)	31.39[c] (7.7)
1,2-di-*O*-hexadecanoyl-3-*O*-[(2-bromoethyl)phosphoryl]-*rac*-glycerol	**26**[f]	64.13 (4.4)	71.01 (8.5)	63.17	65.89 (4.5)	31.25[c] (8.1)

[a] Proton-decoupled 20-MHz ^{13}C NMR spectra were measured in $CDCl_3$–CD_3OD–D_2O (50:50:15, by volume)[3] at 37 ± 1 °C; chemical shifts (δ) are given downfield from Me_4Si and coupling constants in hertz. Additional signals occur at δ 14.18 ± 0.06 (ω CH_3), 22.98 ± 0.07 (ω-1 CH_2), 32.20 ± 0.12 (ω-2 CH_2), and 29.77 ± 0.30 (–$(CH_2)_n$–) for the distal part of the aliphatic moieties. The cis-9 unsaturated compounds **3**, **5**, **6**, **11**, **13**, **20**, and **23** also show the respective olefinic carbon signals at 130.33 ± 0.03 and 130.09 ± 0.09 ppm. C-1′ of *O*-acyl appears at 174.95 ± 0.07 in acyl ethanediol phospholipids, at 175.23 ± 0.04 in acyl 1,3-propanediol phospholipids, and at 174.60 ± 0.06 and 174.19 ± 0.08 in the glycerophospholipids, whereas C-2′ and C-3′ of *O*-acyl give signals at 34.52 ± 0.16 and 25.35 ± 0.16 ppm, respectively. C-1′ of *O*-alkyl is observed at 71.90 ± 0.05 (ethanediol ethers), 71.54 ± 0.04 (propanediol ethers), or 71.06 and 72.16 (glycerol ethers), whereas C-2′ of *O*-alkyl gives a signal at 26.35 ± 0.08. [b] Two-bond ^{13}C–^{31}P couplings ($^2J_{CP}$) are given in parentheses. [c] Three bond ^{13}C–^{31}P couplings ($^3J_{CP}$) are given in parentheses. [d] $^3J_{CP}$ values given for choline derivatives are approximate and were obtained after correcting for ^{13}C–^{14}N couplings (J_{CN} = 3.5 Hz). [e] J_{CN} = 3.5 ± 0.2 Hz. [f] For synthesis, see ref 4. [g] Superimposed. [h] Prepared from the respective alkyl or acyl diol[8] by procedures described in ref 4. [i] Prepared from 1,2-di-*O*-hexadecyl-*rac*-glycerol[9] by procedures described in ref 4. [j] Prepared from 1,2-diolein[10] by procedures described in ref 4. [k] For preparation, see ref 6. [l] Prepared from **13** or **20** of egg yolk, respectively, using phospholipase A_2 hydrolysis.[11] [m] For preparation, see ref 7. [n] Prepared from the respective hydroxy lipids by a modified Hirt and Berchtold synthesis.[5]

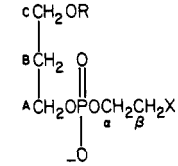
Chart I



1, R = $C_{13}H_{27}$; X = $^+N(CH_3)_3$
16, R = $C_{15}H_{31}$; X = NH_2



2, R = $C_{16}H_{33}$; X = $^+N(CH_3)_3$
3, R = $C_{16}H_{31}$; X = $^+N(CH_3)_3$
4, R = $C_{15}H_{31}CO$; X = $^+N(CH_3)_3$
5, R = $C_{15}H_{29}CO$; X = $^+N(CH_3)_3$
6, R = $C_{17}H_{33}CO$; X = $^+N(CH_3)_3$
17, R = $C_{16}H_{33}$; X = NH_2
22, R = $C_{16}H_{33}$; X = Br
23, R = $C_{17}H_{33}CO$; X = Br



7, R = $C_{16}H_{33}$; X = $^+N(CH_3)_3$
8, R = $C_{15}H_{31}CO$; X = $^+N(CH_3)_3$
18, R = $C_{16}H_{33}$; X = NH_2
24, R = $C_{16}H_{33}$; X = Br
25, R = $C_{15}H_{31}CO$; X = Br

al.[13] and clearly show that the assignments made by others (incorrect C_β and C_C;[14] reversal of C_α and C_A[15]) were in error.

Carbon–Phosphorus and Carbon–Nitrogen Couplings. Table I lists two-bond ^{13}C–^{31}P couplings ($^2J_{CP}$) and three-bond ^{13}C–^{31}P couplings ($^3J_{CP}$) of the phospholipids **1**–**26**, as well as the respective ^{13}C–^{14}N couplings (J_{CN}) of the choline derivatives **1**–**15** as they were measured in $CDCl_3$–CD_3OD–D_2O (50:50:15, by volume). Carbon–nitrogen couplings were not observed for the ethanolamine phospholipids (**16**–**21**) which can be attributed to the asymmetry at the nitrogen nucleus.[16]

The **two-bond carbon–phosphorus couplings** involve the methylene carbon C_A of the phospholipid backbones and the methylene carbon C_α of the polar headgroups (Charts I and II). The $^2J_{CP}$ couplings measured range from 3.5 to 5.9 Hz for these proximal carbons on either side of the phosphorus, values which are similar to those ($^2J_{CP}$ 4.0–6.3 Hz) reported for various nucleotides,[17–21] sugar phosphates[22] and cyclic glycerol acetal phosphates.[23] The precise relationship between the magnitude of two-bond ^{13}C–^{31}P couplings and conformational parameters is presently not well understood.

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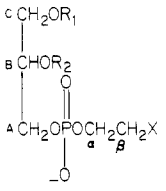
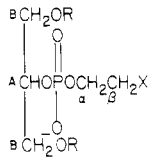
Chart II



9, $R_1 = R_2 = C_{16}H_{33}$; $X = {}^+N(CH_3)_3$
10, $R_1 = R_2 = C_{15}H_{31}CO$; $X = {}^+N(CH_3)_3$
11, $R_1 = R_2 = C_{17}H_{33}CO$; $X = {}^+N(CH_3)_3$
13, R_1 and R_2 = acyl; $X = {}^+N(CH_3)_3$
14, R_1 = acyl; $R_2 = H$; $X = {}^+N(CH_3)_3$
15, $R_1 = R_2 = H$; $X = {}^+N(CH_3)_3$
19, $R_1 = R_2 = C_{15}H_{31}CO$; $X = NH_2$
20, R_1 and R_2 = acyl; $X = NH_2$
21, R_1 = acyl; $R_2 = H$; $X = NH_2$
26, $R_1 = R_2 = C_{15}H_{31}CO$; $X = Br$



12, $R = C_{15}H_{31}CO$; $X = {}^+N(CH_3)_3$

In contrast, **three-bond carbon–phosphorus couplings** are known[18] to be sensitive to changes in dihedral angles between the ^{31}P–O–C and the O–C–^{13}C planes and they respond to angular changes in a fashion that is approximated by the Karplus equation.[24] Three-bond carbon–phosphorus couplings can vary widely (1–10 Hz).[18-23] Large $^3J_{CP}$ values (≥8 Hz) indicate trans coupling, smaller $^3J_{CP}$ values (≤2 Hz) are characteristic for cis/gauche orientation.[20] Three-bond carbon–phosphorus couplings have previously been used to measure the conformation of nucleotides[17-21] and sugar phosphates.[22] They have also been useful in describing prevalent rotamer populations of isomeric cyclic glycerol acetal phosphates as function of ring acetal configuration and phosphate substitution.[23]

The three-bond ^{13}C–^{31}P couplings of phospholipids **1–26** involve the backbone carbons C_B and the headgroup carbons C_β (Charts I and II). Because the β-methylene carbon of the choline derivatives **1–15** simultaneously couples to the choline nitrogen, the $^3J_{CP}$ values given for C_β of choline phospholipids were extracted after correcting for the ^{13}C–^{14}N couplings of 3.5 Hz.

Essentially all phospholipids studied (except **12**; see Table I) show consistently high $^3J_{CP}$ couplings for the backbone carbon C_B which are in the vicinity of 8 Hz (7.8 ± 0.9 Hz). This indicates that phospholipids of these types in solution distinctly prefer a fully extended conformation from the lipid phosphorus toward the lipid backbone in which the backbone C_A–C_B bond would be oriented trans in respect to the O–P bond.[25] Interestingly, the phospholipids with $^3J_{CP}$ couplings for C_B at the lower end of the range (≤7.6 Hz), particularly include structures such as the alkyl phospholipids (**1, 16**), the lysophospholipids (**14, 21**), and some of the diol phospholipids (**4, 7, 18**) which lack an additional alkyl chain at C_B. This suggests that these monochain phospholipids, although largely trans oriented, would permit some additional contribution from gauche conformers because of lessened rotational restrictions about the C_A–O bond due to the absence of an additional long-chain substituent at C_B. Only for the 1,3-diacylglycero-2-phosphocholine **12** an exceptionally small $^3J_{CP}$ coupling of 3.9 Hz was observed, indicating that the C_B carbons in this unnatural phosphatidylcholine isomer are essentially locked in gauche conformation.

The $^3J_{CP}$ couplings of phospholipids **1–26** for the headgroup carbons C_β range from 6.0 to 8.1 Hz (Table I). They are generally higher for the choline (7.5 ± 0.5 Hz) and bromoethanol (7.8 ± 0.3 Hz) derivatives than they are for the ethanolamine phospholipids ($^3J_{CP}$ = 6.4 ± 0.6 Hz). This signifies that the β-carbon of all phospholipids studied essentially assumes trans orientation in respect to the phosphorus and that the contribution from gauche conformers is quite small in the case of the choline (**1–15**) and bromoethanol (**22–26**) compounds. Yet, the lower $^3J_{CP}$ values obtained for the ethanolamine phospholipids (**16–21**) would indicate that the less bulky amino group would allow freer rotation about the C–O bond of the aminoethyl phosphate function and hence would permit some contribution from gauche conformers. These results are in excellent agreement with the predictions derived from quantum-mechanical calculations of the effect of hydration on the conformational properties of choline and ethanolamine phospholipid headgroups. Such calculations predicted general trans orientation for this type of phospholipid headgroup and a somewhat higher gauche contribution in the case of phosphatidylethanolamines.[26]

The consistency of $^2J_{CP}$ and $^3J_{CP}$ couplings which we observed for the respective carbon resonances of a great variety of phospholipids in chloroform–methanol–water (50:50:15, by volume) indicates that the backbone and headgroup carbons of these phospholipids (except **12**) in solution would orient themselves in a rather unobstructed fashion in which C_B of the backbone and C_β of the headgroup predominantly assume trans disposition in respect to the phosphorus. These findings complement rather well the data that have recently been obtained through NMR studies on phosphatidylcholines and lysophosphatidylcholine analogues in methanolic or aqueous dispersions.[27] They are also consistent with the results of X-ray structural analyses on phosphatidylcholine crystals[28] and with predicitions based on computational conformation analysis.[29]

Because two-bond and three-bond carbon–phosphorus couplings of phospholipids in solution have now become rather predictable, the couplings can hence be used to facilitate chemical shift assignments in phospholipid spectra on the basis of the absence or presence of carbon–phosphorus and carbon–nitrogen couplings *and* on the basis of the *magnitude* of $^2J_{CP}$ and $^3J_{CP}$ values. While small carbon–phosphorus couplings (averaging 4.9 Hz) would in general be characteristic for the $^2J_{CP}$ couplings of C_A and C_α, larger values (averaging 7.6 Hz) would indicate $^3J_{CP}$ couplings of C_B and C_β. C_B and C_β of choline phospholipids can readily be distinguished from each other by virtue of C_β being also spin coupled to ^{14}N. C_C of the backbone, in turn, is not coupled to phosphorus.

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Synthesis of 3β,29-Dihydroxystigmasta-5,24(28)(*E*)-dien-7-one

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The synthesis of 3β,29-dihydroxystigmasta-5,24(28)(*E*)-dien-7-one from 3β-acetoxy-22,23-dinorcholenaldehyde has been achieved in an overall yield of 7%. Wittig reaction of the aldehyde and diethyl (3-methyl-2-oxobutyl)phosphonate anion gave 3β-acetoxycholesta-5,22-dien-24-one which was hydrogenated selectively, and the saturated ketone was allowed to react with the anion of diethyl (cyanomethyl)phosphonate. The resulting nitrile was reduced via the aldehyde to 29-hydroxyfucosterol by using DIBAL-H. Acetylation of this diol and oxidation with chromium trioxide–3,5-dimethylpyrazole afforded the 7-ketone. Mild hydrolysis of the acetate groups completed the synthesis.

We recently reported the identification of 3β,11α,15β,29-tetrahydroxystigmasta-5,24(28)(*E*)-dien-7-one, 3β-isobutyrate ("dehydrooogoniol-1"), a female-activating hormone of the aquatic fungus *Achlya*.[1] The substance appears to be the most active of the oogoniols so far isolated, and we have undertaken synthetic studies to confirm the structure and to develop a method for securing adequate quantities of the steroid for further biological investigation. The model steroid 3β,29-dihydroxystigmasta-5,24(28)(*E*)-dien-7-one (29-hydroxy-7-oxofucosterol) was selected as our first synthetic target for two reasons. First, we felt that it would be readily accessible from 3β-acetoxy-22,23-dinorcholenaldehyde by a series of straightforward reactions. These could later be applied to the synthesis of dehydrooogoniol-1 itself by starting from a suitably functionalized aldehyde. Second, we were interested in determining if the model steroid which lacks the hydroxyl groups at C-11 and C-15 in dehydrooogoniol-1 would show any biological activity. This paper describes the synthesis of the model steroid (**12**) from the dinorcholenaldehyde (**1**).

Conversion of the dinorcholenaldehyde (**1**) to 3β-acetoxycholesta-5,22(*E*)-dien-24-one (**2**) was reported earlier.[2,3] While the yield obtained in the Wittig reaction was high, the conditions were rather severe (reactants in Me_2SO heated at 95 °C for 65 h). We have found that by using the more reactive phosphonate anion rather than phosphorane, a high yield of dienone **2** can be obtained by refluxing for 1.5 h in tetrahydrofuran. Catalytic hydrogenation of dienone **2** with 10% Pd on $BaSO_4$ afforded 24-oxocholesterol acetate (**3**) in 93% yield.

A number of ways were available for completing the fucosterol skeleton. Grignard reaction of vinylmagnesium bromide and ketone **3** gave a mixture of saringosterol and its acetate **4**. Acetylation of the mixture with acetic anhydride–pyridine gave a quantitative yield of **4**. Treatment of acetate **4** with a large excess of pyridinium chlorochromate converted the tertiary allylic alcohol moiety to the unsaturated aldehyde **5** in only moderate yield. An alternative route involved reaction of ketone **3** with the anion of diethyl (cyanomethyl)phosphonate which yielded the unsaturated nitrile as a mixture of isomers (ratio of 3:1 *E*/*Z*). The isomers could be distinguished by the signal for the C-28 vinyl proton which occurred at δ 5.01 in the *Z* isomer and at δ 5.08 in the *E* isomer. The signal for the C-25 methine proton in the *Z* isomer occurred at δ ~3.1 while that for the *E* isomer overlapped other signals at δ ~2.3.[4] The isomers had different retention times on gas chromatography. The mixture of nitriles (**6**) was treated with diisobutylaluminum hydride (DIBAL-H) followed by dilute acid to give, after reacetylation, the corresponding unsaturated aldehydes in high yield. These could be separated by chromatography.

Reduction of aldehyde **5** (mixture of isomers) with sodium borohydride furnished the 29-hydroxy derivative (**7**) together with some of the saturated alcohol resulting from 1,4-reduction. A better yield of alcohol **7** was obtained by reducing aldehyde **5** with DIBAL-H, and there was no evidence of 1,4-reduction.

Attempts were made to introduce the carbonyl function at position C-7 in the diol **7** by photooxygenation to the 5α-hydroperoxide followed by rearrangement to the 7α-hydroperoxide and loss of water. This method had been used successfully in the synthesis of antheridiol and deoxyantheridiol.[2] However, in the case of the diol **7** a complex mixture was formed because the side-chain double bond was also attacked by singlet oxygen. It has been reported that the presence of a hydroxyl group at an allylic position retards photooxygenation of the double bond, and conversion of the alcohol to an acetate or benzoate completely suppresses such a reaction.[5] The deactivation was attributed to electron withdrawal by the allylic substituent. The C-24(28) double bond in the diol **7** and in the corresponding diacetate was, however, very susceptible to attack by singlet oxygen, and a mixture of products was obtained in each caase.

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