BIOORGANIC CHEMISTRY **16,** 111–123 (1988)

Negative Ion Fast Atom Bombardment Mass Spectrometry of Isoprenoid Diphosphates and Related Analogs

V. JO DAVISSON, THOMAS R. SHARP,[1] AND C. DALE POULTER[2]

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

Received April 28, 1987

Negative ion fast atom bombardment mass spectrometry (FABMS) was used to obtain mass spectra of the naturally occurring isoprenoid metabolites isopentenyl diphosphate (**1**), dimethylallyl diphosphate (**5**), geranyl diphosphate (**3**), farnesyl diphosphate (**6**), and presqualene diphosphate (**8**). Mass spectra were also recorded for several synthetic analogs, including methylene-bridged diphosphonates **9** and **10**, bisdiphosphates **12–14**, and nitrogen-containing analogs **15–18**. The ammonium salts of these materials gave molecular ions at $[M-H]^-$ for the acid forms (M) of the diphosphate esters. Spectra of the sodium salts of isopentenyl diphosphate (**1**) and geranyl diphosphate (**3**) showed predominant peaks at *m/z* 267 $[M + Na-2H]^-$ and 335 $[M + Na-2H]^-$, respectively, along with several diagnostic fragment ions at *m/z* 177 ($H_3P_2O_7$), 159 (HP_2O_6), and 97 (H_2PO_4) from the diphosphate moiety. The molecular ion of isopentenyl diphosphate (**1**) (*m/z* 245) was readily observed with 4 μg of compound. Thus, detection at the microgram level should be possible for disphosphates whose molecular ions do not overlap with peaks from the matrix. These studies indicate that negative ion FABMS will be useful for the analysis of isoprenoid diphosphate intermediates in biosynthetic and enzyme mechanism studies. © 1988 Academic Press, Inc.

INTRODUCTION

The isoprenoid biosynthetic pathway is ubiquitous and employs diphosphate esters as intermediates in all organisms to build a wide variety of isoprenoid hydrocarbon chains (*1*). The end products of the pathway constitute a diverse array of metabolites, which includes sterols, carotenoids, respiratory quinones, dolichols, mono-, di-, and sesquiterpenes, and zeatins (*1, 2*). Biosynthetic studies of the intermediate stages of isoprenoid metabolism have been limited by the unavailability and chemical instability of the diphosphate ester substrates. These compounds contain either 3,3-dialkylallylic (*3*) or allyl-substituted cyclopropylcarbinyl (*4*) moieties, which generate potent electrophiles upon cleavage of the reactive carbon–oxygen bond to the diphosphate group. Reactivity is enhanced upon protonation of the diphosphate (*3*). As a result, isoprenoid diphosphates are usually analyzed after enzymatic hydrolysis of the diphosphate moiety and conversion of the resulting alcohols to nonvolatile derivatives (*5*). Recent advances in the synthesis and isolation of this class of compounds provide viable sources of materials (*6*). However, analysis of microgram quantities of isoprenoid diphos-

---

[1] Present address: Department of Chemistry, Texas A & M University, College Station, TX 77843.

[2] To whom all correspondence should be addressed.

0045-2068/88 $3.00

Copyright © 1988 by Academic Press, Inc.
All rights of reproduction in any form reserved.

phates usually requires chromatographic separations of radiolabeled materials comixed with authentic standards to verify mobilities.

The advent of "soft" ionization methods (*7*) in mass spectrometry opened new avenues for analysis of a variety of molecules with high polarity and low volatility, including phosphate esters. Both positive and negative ion fast atom bombardment (FAB) techniques (*8*) have been used with a high degree of success (*9–18*). Mass spectra of naturally occurring cyclic nucleotides (*19*) and polyphosphorylated inositols (*20*) were obtained using this technique. In addition, FAB mass spectral analyses of adenosine triphosphate and its derivatives (*21, 22*) were recently employed to study enzyme reaction mechanisms. The failure of other ionization methods and the recent publication of a negative ion FAB mass spectrum for retinyl phosphate (*23*) prompted us to explore the technique for adaptation to a wide variety of isoprenoid diphosphates. We now report our success with this class of molecules using negative ion FAB mass spectrometry (FABMS) (*24*).

EXPERIMENTAL

Detailed preparations of isopentenyl (**1**), dimethylallyl (**5**), geranyl (**3**), and farnesyl (**6**) diphosphates and analogs **9–11** and **15** were recently reported (*6*). Full accounts of the syntheses of presqualene diphosphate (**8**) (*25*) and compounds **16–18** are available (*26*). Preparations of compounds **12–14** will be described in a forthcoming paper (*27*). Deuterated analog **7** was prepared from [1-^{2}H]farnesol obtained by oxidation of *E,E*-farnesol (Aldrich Chemical Co.) with mangenese dioxide and reduction of the resulting aldehyde with sodium borodeuteride. The sodium salts of isopentenyl diphosphate (**2**) and geranyl diphosphate (**4**) were prepared by cation exchange chromatography on Dowex AG50W-X8 (sodium form, Bio-Rad Laboratories). A typical exchange involved dissolving the ammonium salts in deionized water and passage of the solution through a 1 by 5-cm column of ion exchange resin. Twenty milliliters of eluant was collected and lyophilized to give the sodium salts as white solids in 92–98% yields. All samples used in this study were judged pure by thin-layer chromatography (TLC) on cellulose and by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy.

Mass spectra were obtained with a VG Analytical 7070-E magnetic sector instrument operated at −5.0 kV accelerating potential and 1000 resolving power. FAB samples were bombarded with a neutral argon beam at 6 keV produced by an Ion Tech saddle field gun operated at 1.0 mA. The magnetic field was routinely scanned at 20 s/decade. The VG DS 2050 data system was calibrated with 85% phosphoric acid or with concentrated sulfuric acid (*28*).

Solid ammonium or sodium salts of the diphosphates were dissolved in liquid matrices and 2 to 3-μl portions were applied to the FAB probe. Matrices consisted of glycerol, a 9 : 1 (v/v) mixture of glycerol and 15-crown-5 (*29*) or with 5–10% 0.1 N NaOH in either of the two matrices (*30*). Peaks for ions from the matrix were assigned from FAB spectra of salts dissolved in [1,2,3,-^{2}H$_5$]glycerol (98% ^{2}H$_5$, Cambridge Isotopes). Estimates of the number of exchangeable hydrogens were obtained by independent analyses in [O^{2}H]$_3$-glycerol (98% ^{2}H$_3$, KOR Isotopes)

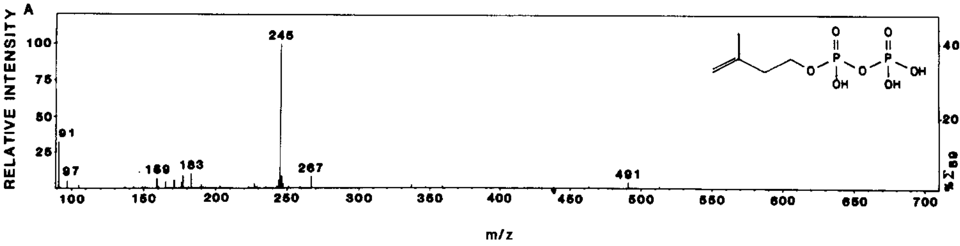


FIG. 1A. Negative ion FAB mass spectrum of isopentenyl diphosphate ammonium salt **1** in a glycerol/15-crown-5 matrix.

(*31*). A 9 : 1 : 0.1 (v/v/v) mixture of 15-crown-5, water, and glycerol was used as the solvent and matrix for sensitivity studies. A stock solution of isopentenyl diphosphate (**1**) 5 mg/ml in the matrix–solvent was prepared for serial dilutions. Three-microliter samples of each dilution were applied to the FAB probe.

RESULTS AND DISCUSSION

A preliminary inspection of the positive ion FAB mass spectra of **1** and **3** did not yield interpretable spectra. In contrast, the ammonium salts of both diphosphates gave simple negative ion FAB mass spectra with intense molecular ions. These dramatic differences have been noted in reports of positive ion FAB mass spectra of compounds with phosphate moieties that lack molecular positive ions or have reduced sensitivity when compared to the negative ion spectra (*11, 20*).

Isopentenyl diphosphate was chosen for the initial studies because it is more stable than allylic or cyclopropylcarbinyl derivatives. Both the ammonium and sodium salts of compounds **1–4** (Table 1) were examined in several matrices. Ammonium salt **1** (Fig. 1A) gave a molecular ion at *m*/*z* 245 as the base peak in a glycerol matrix containing 15-crown-5. This ion was assigned to a $C_5H_{11}P_2O_7$ species, which corresponds to the monoanion $[M-H]^-$ of the free acid (M) of isopentenyl diphosphate (**1**). As can be seen in Fig. 2A, the molecular ion for geranyl diphosphate (**3**) $[M-H]^-$ was also the most intense peak in the mass spectrum. In the same matrix, the sodium salts (**2** and **4**) of these two diphosphates (Table 1) gave prominent molecular ions for the monosodium–monoprotio species $[M + Na-2H]^-$ (Figs. 1B and 2B and Table 1). Under these conditions, the total

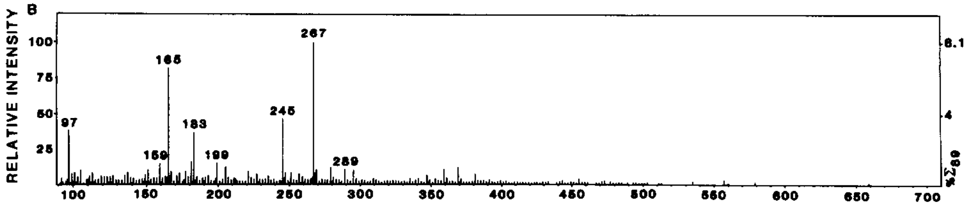
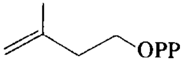
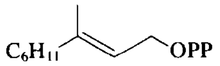
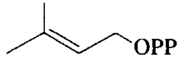
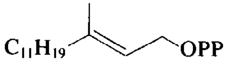


FIG. 1B. Negative ion FAB mass spectrum of isopentenyl diphosphate sodium salt **2** in a glycerol/15-crown-5 matrix.

TABLE 1

Compound	Structure[a]	Salt form	Mol. wt. free acid	Major ions (−) FABMS[b]
1	 Isopentenyl diphosphate/ glycerol/15-crown-5	$3NH_4^+$	246	491 (4.2) $C_{10}H_{23}O_{14}P_4$, 267 (7.9), 245 (100) $C_5H_{11}O_7P_2$, 227 (2.8) $C_5H_9O_6P_2$, 183 (12.4) glycerol, 177 (8.9) $H_3O_7P_2$, 171 (6.0), 165 (4.5) $C_5H_{10}O_4P$, 159 (6.7) HO_6P_2, 97 (5.1) H_2O_4P, 91 (36.7) glycerol
2		$3Na^+$		535 (2.0) $C_{10}H_{21}Na_2O_{14}P_4$, 295 (7.4), 289 (8.2) $C_5H_9Na_2O_7P_2$, 279 (8.9), 267 (72.4) $C_5H_{10}NaO_7P_2$, 245 (33.7) $C_5H_{11}O_7P_2$, 221 (69) $HNaO_7P_2$, 205 (9.2) glycerol · Na, 199 (11.8) $H_2NaO_7P_2$, 183 (26.5) glycerol, 181 (12.0) NaO_6P_2, 177 (6.9) $H_3O_7P_2$, 165 (59.6) $C_5H_{10}O_4P$, 159 (10.9) HO_6P_2, 97 (27.7) H_2O_4P, 91 (100) glycerol
3	 Geranyl diphosphate/ glycerol/15-crown-5	$3NH_4^+$	314	627 (5.1) $C_{20}H_{39}O_{14}P_4$, 313 (100) $C_{10}H_{19}O_7P_2$, 295 (7.8) $C_{10}H_{17}O_6P_2$, 277 (7.1) $C_{10}H_{15}O_5P_2$, 243 (7.1), 177 (44) $H_3O_7P_2$, 159 (47) HO_6P_2, 97 (8.3) H_2O_4P
4		$3Na^+$		591 (5.2), 489 (5.0), 449 (5.7), 437 (15.5), 427 (8.5), 415 (13.0), 393 (6.1), 357 (6.7) $C_{10}H_{17}Na_2O_7P_2$, 335 (95.7) $C_{10}H_{18}NaO_7P_2$, 313 (69.1) $C_{10}H_{19}O_7P_2$, 295 (13.1) $C_{10}H_{17}O_6P_2$, 233 (100) $C_{10}H_{18}O_4P$, 221 (7.2) $HNa_2O_7P_2$, 205 (7.3) glycerol · Na, 199 (25.8) $H_2NaO_7P_2$, 181 (25.3) NaO_6P_2, 177 (18.9) $H_3O_7P_2$, 159 (32.4) HO_6P_2, 153 (10), 97 (48.4) H_2O_4P, 91 (81) glycerol
5	 Dimethylallyl diphosphate/glycerol	$3NH_4^+$	246	491 (4.1) $C_{10}H_{23}O_{14}P_4$, 337 (5.1), 245 (100) $C_5H_{11}O_7P_2$, 183 (19.2) glycerol, 177 (26.9) $H_3O_7P_2$, 165 (5.5) $C_5H_{10}O_4P$, 159 (12.4) HO_6P_2, 97 (5.8) H_2O_4P, 91 (44.9) glycerol
6	 Farnesyl diphosphate/glycerol	$3NH_4^+$	382	397 (6.8), 381 (62.3) $C_{15}H_{27}O_7P_2$, 293 (4.9), 275 (7.0) glycerol, 183 (90.3) glycerol, 177 (22.4) $H_3O_7P_2$, 159 (18.8) HO_6P_2, 97 (11) H_2O_4P, 91 (100) glycerol

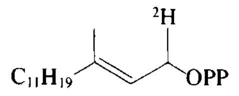
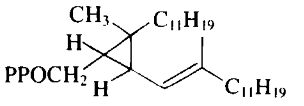
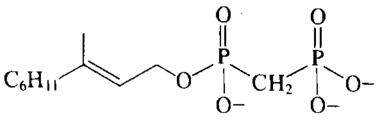
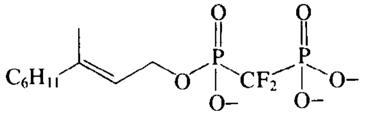
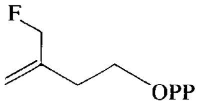
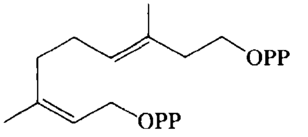
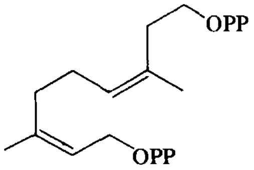
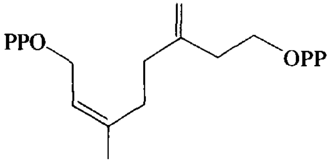
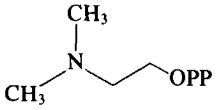
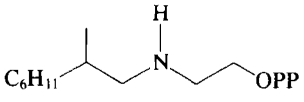
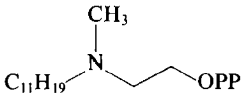
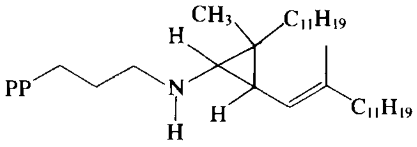
7	 11-[^{2}H]Farnesyl diphosphate/glycerol	$3NH_4^+$	383	382 (56.7) $C_{15}H_{28}O_7P_2$, 364 (3.8) $C_{15}H_{26}O_6P_2$, 277 (6.6) 261 (6.1), 239 (4.1), 177 (52.4) $H_3O_7P_2$, 159 (100) HO_6P_2, 97 (13.4) H_2O_4P, 91 (6.0) glycerol
8	 Presqualene diphosphate/glycerol	$3NH_4^+$	586	585 (25.1) $C_{30}H_{51}O_7P_2$, 277 (4.1), 275 (11.5) glycerol, 190 (11), 183 (90) glycerol, 181 (11.8) glycerol, 177 (14.4) $H_3O_7P_2$, 159 (30) HO_6P_2, 153 (5.9), 151 (7.7), 97 (15.7) HO_4P, 91 (100) glycerol
9	 Glycerol	$3NH_4^+$	312	447 (5.0) $C_{21}H_{37}O_6P_2$, 353 (7.2), 311 (83) $C_{11}H_{21}O_6P_2$, 293 (38.3) $C_{11}H_{19}O_5P_2$, 275 (7.9) glycerol, 241 (11), 223 (7), 175 (31.5) $CH_5O_6P_2$, 159 (8.8) HO_6P_2, 157 (100) $CH_3O_5P_2$, 139 (8.4), 91 (7.7) glycerol
10	 Glycerol	$3NH_4^+$	348	483 (7.5) $C_{21}F_2H_{35}O_6P_2$, 347 (100) $C_{11}F_2H_{19}O_6P_2$, 329 (7.9) $C_{11}F_2H_{17}O_5P_2$, 267 (27.7) $C_{11}F_2H_{18}O_3P$, 233 (18.9) $C_{10}H_{18}O_4P$, 211 (19) $CF_2H_3O_6P_2$, 197 (10.8), 193 (30) $CFH_4O_6P_2$, 159 (12.5), 131 (18.6) $CF_2H_2O_3P$, 129 (16.5), 97 (16.1) H_2O_4P
11	 Glycerol	$3NH_4^+$	264	263 (100) $C_5FH_{10}O_2P_2$, 245 (4.8) $C_5FH_8O_6P_2$, 183 (11.8) Glycerol, 177 (5.3) $H_3O_7P_2$, 159 (9.3) HO_6P_2, 97 (6) H_2O_4P, 91 (28) glycerol
12	 Glycerol	$6NH_4^+$	504	503 (44.9) $C_{11}H_{23}O_{14}P_4$, 423 (9.3) $C_{11}H_{22}O_{11}P_3$, 417 (4.8) $C_{11}H_{19}O_7P_2$ + glycerol, 325 (6.0) $C_{11}H_{19}O_7P_2$, 275 (4.3) glycerol, 258 (5.7), 190 (9.8), 183 (65.3) glycerol, 181 (22.9) glycerol, 177 (75.3) $H_3O_7P_2$, 165 (6.6), 159 (55.9) HO_6P_2, 151 (13), 121 (7.7), 97 (36.7) H_2O_4P, 91 (100) glycerol

TABLE 1—*Continued*

Compound	Structure[a]	Salt form	Mol. wt. free acid	Major ions (−) FABMS[b]
13	 Glycerol	$6NH_4^+$	504	503 (44.9), $C_{11}H_{23}O_{14}P_4$, 423 (16.1), $C_{11}H_{22}O_{11}P_3$, 417 (6.6) $C_{11}H_{19}O_7P_2$ + glycerol, 387 (5.1), 327 (8.7), 325 (11) $C_{11}H_{19}O_7P_2$, 258 (7.9), 190 (10.9), 183 (68.4) glycerol, 181 (11.2) glycerol, 177 (46.8) $H_3O_7P_2$, 159 (39.5) HO_6P_2, 151 (5.6), 97 (22.7) H_2O_4P, 91 (100) glycerol
14	 Glycerol	$6NH_4^+$	490	489 (9) $C_{10}H_{21}O_{14}P_4$, 275 (8.6) glycerol, 183 (100) glycerol, 181 (17) glycerol, 177 (15.4) $H_3O_7P_2$, 165 (14.7), 159 (13.2) HO_6P_2, 151 (8.4), 97 (9.8) H_2O_4P, 91 (98) glycerol
15	 Glycerol	$3NH_4^+$	249	275 (7.3) glycerol, 248 (19.7) $C_4H_{12}NO_7P_2$, 183 (82.5) glycerol, 181 (8.2) glycerol, 177 (2.5) $H_3O_7P_2$, 153 (12.1), 151 (4.0), 91 (100) glycerol

16	 Glycerol	$3NH_4^+$	345	344 (3.2) $C_{11}H_{24}NO_7P_2$, 275 (6.3) glycerol, 183 (90) glycerol, 181 (7) glycerol, 151 (3.9), 91 (100) glycerol.
17	 Glycerol	$3NH_4^+$	385	384 (100) $C_{14}H_{28}NO_7P_2$, 275 (4.3) glycerol, 247 (5.5), 234 (3.2) $C_3H_{10}NO_7P_2$, 203 (6.7), 190 (3.9), 183 (59) glycerol, 181 (6.8) glycerol, 177 (22.5) $H_3O_7P_2$, 171 (26), 159 (17.4) HO_6P_2, 153 (15.5), 151 (4.4), 127 (4.6), 97 (10.8) H_2O_4P, 91 (97.8) glycerol
18	 Glycerol	$3NH_4^+$	613	612 (22.4) $C_{32}H_{56}NO_6P_2$, 275 (3.7) glycerol, 245 (3.2), 201 (5.1), 187 (5.7), 183 (36.7) glycerol, 177 (8.6), 174 (7.2), 159 (14.4) HO_6P_2, 153 (28), 143 (6), 97 (29.1) H_2O_4P, 91 (100) glycerol

---

[a] C_{11} = 4-Methyl-3-penten-1-yl. $C_{11}H_{19}$ = *E*-4,8-Dimethyl-3,7-nonadiene-1-yl.

[b] All empirical formulas are assigned from low resolution *m/z* values. Normalization is to the most intense peak in the spectrum, regardless of its origin.

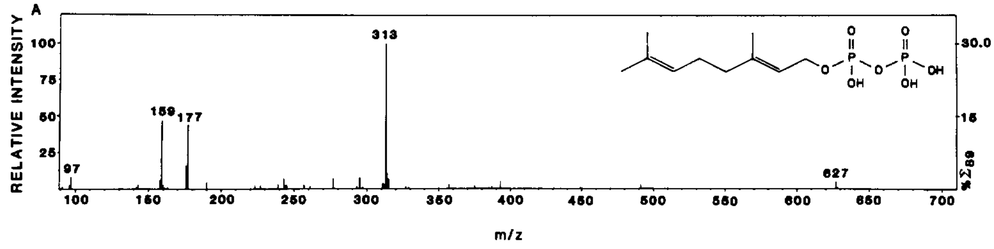


FIG. 2A. Negative ion FAB mass spectrum of geranyl diphosphate ammonium salt **3** in a glycerol/15-crown-5 matrix.

ion current was also distributed among several fragment ions. In contrast, the FAB mass spectra of other organic and organometallic systems are not significantly influenced by the nature of the counterion (*33*, *34*). The matrix ions, especially prevalent for the sodium salts or when sodium hydroxide was added, were less prominent when 15-crown-5 was used as a matrix cosolvent (*29*). The large ion current associated with $[M-H]^-$ for the ammonium salts suggested that the diprotium species, which arises from disproportionation of ammonium ions under high vacuum, would give a higher degree of sensitivity.

A series of negative ion FAB mass spectra were obtained for compounds **1–4** to verify assignments for the matrix peaks and the number of active hydrogens in each ion. When the matrix consisted of $[1,2,3\text{-}^2H_5]$glycerol those peaks from glycerol shifted to higher mass as expected and those due to diphosphate did not shift. Another set of spectra were obtained in $[O^2H]_3$-glycerol as a matrix and showed appropriate shifts for the matrix peaks. In addition, the number of active hydrogens in the diphosphate molecular and fragment ions was deduced from the isotopically induced shifts (*31*).

Several important common features were noted in the negative ion FAB mass spectra of compounds **1–4**. Major structural fragments were minimal for the ammonium salts. A comparison of the spectra for the homoallylic (**1**) and allylic (**3**) diphosphates showed substantial differences in the intensities for the major diphosphate fragments at *m*/*z* 177 $[H_3P_2O_7]^-$, which resulted from cleavage of the carbon–oxygen bond in each molecule. This ion contributed a substantially larger percentage to the total ion current for **3**. An increase in carbon–oxygen bond heterolysis for the allylic diphosphate is consistent with the reactivities of allylic

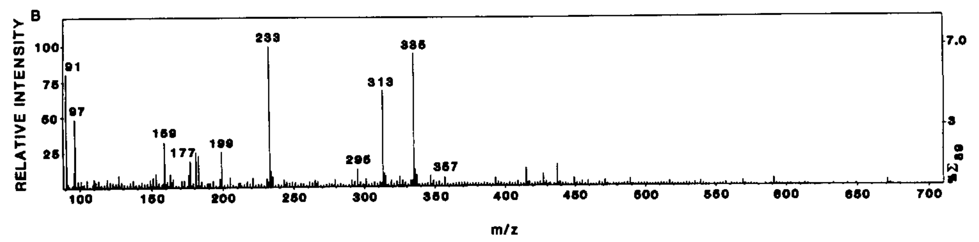


FIG. 2B. Negative ion FAB mass spectrum of geranyl diphosphate sodium salt **4** in a glycerol/15-crown-5 matrix.

and homoallylic systems (*3*). A peak was observed at $[2M-H]^-$ for compounds **1**–**3** and **5**, apparently from a hydrogen-bonded dimeric species. In addition, **4** (Fig. 2B) gave a dimeric peak at *m/z* 671 for $[2M + 2Na-3H]^-$.

Similar species have been reported for other phosphorylated materials (*16, 23*). Another common fragmentation was loss of water from the molecular ions as indicated by peaks at *m/z* 227 for isopentenyl diphosphate (**1**) and *m/z* 295 for geranyl diphosphate (**3**). In addition, there was loss of water from the diphosphate ion indicated by *m/z* 159 (*m/z* 177 − 18, ammonium salts) and 181 (*m/z* 199 − 18, sodium salts). For sodium salts **2** and **4**, the major molecular ions were the $[M + Na-2H]^-$ species, with significant contributions from the $[M-H]^-$ ions. Fragmentation of the two phosphorous–oxygen bonds was also a major mode of decomposition for the sodium salts as indicated by the major peaks at *m/z* 165 and 233, respectively. The spectrum of 4 also contained several unknown ions at molecular weights higher than that of the molecular ion.

The ammonium salts of naturally occurring isoprenoid diphosphates **5**–**8** all gave spectra similar to those for **1** and **3** with molecular ions at $[M-H]^-$ for the free acid forms. In addition, each compound gave phosphate-containing fragments at *m/z* 177 $[H_3P_2O_7]^-$, 159 $[HP_2O_6]^-$, and 97 $[H_2PO_4]^-$ characteristic of rupture of the carbon–oxygen bond (*35*). The reactivity of allylic diphosphate **5** relative to its homoallylic isomer **2** is reflected in the larger ion current for the *m/z* 177 fragment in the FAB mass spectrum of **5** (*3*). The molecular ion of **7** $[M-H]^-$ is shifted by one mass unit as expected for substitution of a single hydrogen atom by deuterium, while the mass/charge ratios of the phosphate fragments do not change. The major features of the FAB mass spectrum of the cycloprophycarbinyl derivative presqualene diphosphate **8** (Fig. 3), with a strong molecular ion (*m/z* 585) and phosphate-containing fragments (*m/z* 177, 159, and 97), are similar to those of acyclic isoprenoids **1**–**7**. This observation is consistent with the comparable reactivities of the allylic and cyclopropylcarbinyl isoprenoids (*3, 4*). The characteristic spectra obtained for **1**–**8** suggest that FAB techniques can be used to detect higher isoprenoid mono- and diphosphates, such as dolichol, as well.

In addition to the natural isoprenoids, the FAB mass spectra of several synthetic analogs were obtained. Although these compounds were previously characterized by other spectroscopic techniques, the results reported in Table 1 are the first examples of a mass analysis. In all cases, ammonium salts were studied in glycerol matrices. Positive ion FAB of potassium salts of derivatives of methane-

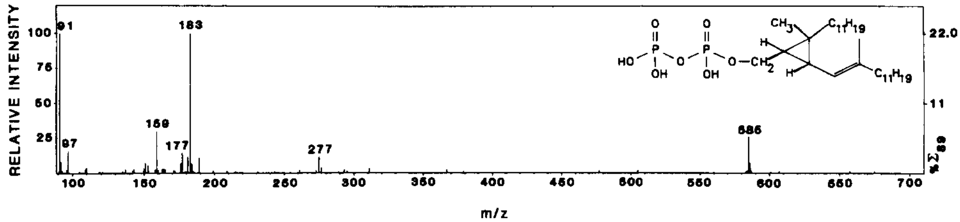
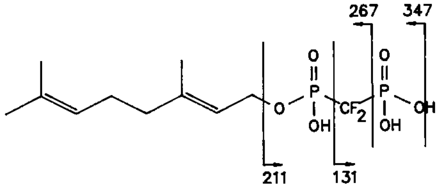


FIG. 3. Negative ion FAB mass spectrum of presqualene diphosphate **8** (20 nmol) in a glycerol matrix.

diphosphonic acids substituted at the bridging methylene carbon in a glycerol matrix was reported to give spectra that were relatively free of interfering peaks (*17*). This procedure cannot be applied directly to isoprenoid methanediphosphonates **9** and **10** because of the instability of the carbon–oxygen linkage in the positive ions. However, negative ion FAB spectra of the ammonium salts of the analogs were qualitatively similar to those obtained for diphosphate **3**, with molecular ions at $M\text{–}H^-$ for the free acids. Methanediphosphonates **9** and **10** also had fragment ions at *m/z* 175 $[CH_5P_2O_7]^-$ or 211 $[CH_3F_2P_2O_6]^-$ for the diphosphonate moieties. In addition, **10** gave substantial peaks at *m/z* 267 $[C_{11}H_{18}F_2PO_2]^-$ and 131 $[CH_2F_2PO_3]^-$, which resulted from rupture of a carbon–phosphorus bond (see Drawing 1). Carbon–phosphorus fragmentation of the negatively charged ions from **10** is probably promoted by the strong electron-withdrawing atoms (*36*) since similar fragments are not seen in the negative ion FAB spectrum of **9**. FABMS was also useful for detection of small amounts of previously undetected symmetric diphosphonate diesters formed during the synthesis of **9** and **10**. These derivatives gave small peaks at *m/z* 447 (6.0% of the molecular ion) and 485 (7.5% of the molecular ion), respectively.

Compounds **12–14** represents a unique class of phosphorylated materials for which verification of the bisdiphosphate ester moieties had been obtained only from ^{31}P NMR spectroscopy (*32*). The negative ion FAB mass spectra for the ammonium salts further substantiate their structures. Molecular ions for compounds **12** and **13** were observed at *m/z* 503 $[M\text{–}H]^-$ corresponding to the acid form of the bisdiphosphates ($C_{11}H_{23}P_4O_{14}$). Fragment ions were more extensive than those observed for diphosphates, with peaks at *m/z* 423 $[M\text{–}H\text{–}HPO_3]^-$, 325 $[M\text{–}H\text{–}H_4P_2O_7]^-$, 177 $[H_3P_2O_7]^-$, and 159 $[HP_2O_6]^-$ representing the major losses (Fig. 4 and Table 1) (*19*). Fragmentation of the two phosphorous–oxygen bonds appeared substantial in these cases. Use of $[O^2H]_3$-glycerol aided in the assignment of these ions. Mass shifted ions at *m/z* 508, 426, 421, 327, 180, and 160 revealed the number of exchangeable hydrogens in each ion and, hence, states of phosphorylation. The peak at *m/z* 325 $[M\text{–}H\text{–}H_4P_2O_7]^-$ indicates that expulsion of neutral diphosphate is possible with the negative charge remaining with the carbon-bound diphosphate. Bisdiphosphates **12** and **13** gave a peak at *m/z* 417 $[M\text{–}H\text{–}H_4P_2O_7 + \text{glycerol}]^-$ consistent with replacement of a diphosphate moiety by glycerol. A substitution of this type is typical of the solvolysis behavior of allylic diphosphates (*3*). Because of these properties, it is important that the matrix be neutral or alkaline. Compound **14**, because of a limited amount of sample, required analysis at the 5 to 6-μg (9 nmol) level. Despite a high matrix background, a



DRAWING 1

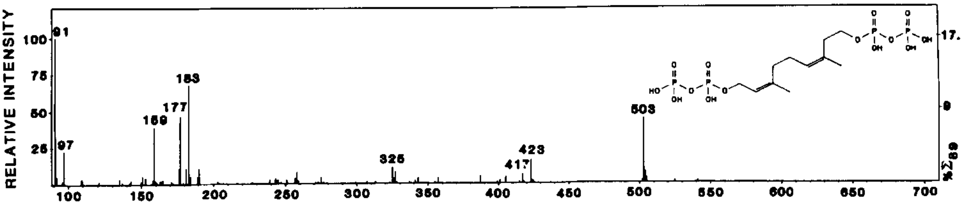


FIG. 4. Negative ion FAB mass spectrum of bisdiphosphate ammonium salt **13** in a glycerol matrix.

molecular ion at *m/z* 489 [M–H]$^-$ was observed along with a peak at *m/z* 177 with the appropriate intensity for carbon–oxygen bond cleavage.

Isoprenoid ammonium analogs **15–18** also gave structurally useful negative ion FAB mass spectra. As shown in Fig. 5 and Table 1, the prominent molecular ions for this subclass of molecules correspond to the deprotonated form of the amino acid. Analogs **15** and **16** gave few peaks in addition to those from the molecular ion and glycerol. Although the spectra for **17** and **18** are somewhat more complex, characteristic phosphate fragments appear at *m/z* 177 $[H_3P_2O_7]^-$, 159 $[HP_2O_6]^-$, 97 $[H_2PO_4]^-$ for **17**, and 97 $[H_2PO_4]^-$ for **18**.

The sensitivity of negative ion FABMS toward isoprenoid diphosphates was evaluated for the ammonium salt of **1** in a matrix consisting of 15-crown-5, water, and glycerol whose viscosity was sufficiently low to permit an accurate serial dilution of the sample. Spectra from the matrix were devoid of peaks at *m/z* 245, where the molecular ion of **1** occurs. Addition of 15 μg (50 nmol) of **1** to the FAB target gave a spectrum in which the *m/z* 245 peak was base peak. At a loading of 7.5 μg (25 nmol), the intensity of the *m/z* 245 peak was still 60% of the most intense peak in the spectrum at *m/z* 193 from 15-crown-5. When the sample of **1** was reduced to 3.75 μg, the intensity of the peak at *m/z* 245 was 37% of the *m/z* 193 peak, although the base peak had shifted to the glycerol dimer at *m/z* 183. A substantial increase in the glycerol peaks was observed as the amount of diphosphate in the matrix decreased. By appropriate choice of matrix conditions, detection of as little as 1 μg of an isoprenoid diphosphate should be possible. These levels of detection are similar to those reported for several different nucleotides (*19*).

CONCLUSIONS

Negative ion FABMS of the ammonium salts of isoprenoid diphosphates and related analogs gave strong molecular ions for the diprotonated forms of the

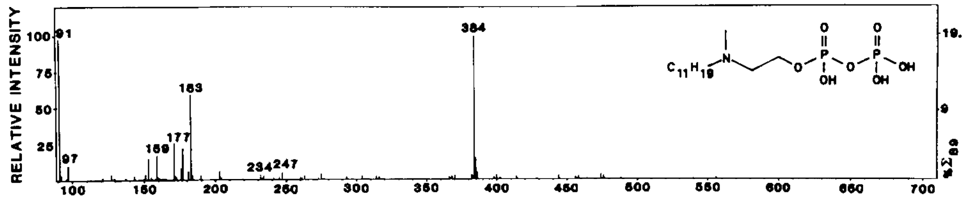


FIG. 5. Negative ion FAB mass spectrum of amine farnesyl analog ammonium salt **17** (26 nmol) in a glycerol matrix.

diphosphate esters using glycerol or 15-crown-5/glycerol matrices. Molecular ions were also seen for the corresponding sodium salts at $[M + Na-2H]^-$, although the ion current was generally reduced due to solute ions. Careful inspection of the spectra revealed no evidence for multiply charged negative ions even for bisdiphosphates **12–14**. The major fragments in the negative ion FAB spectra resulted from clevage of the carbon–oxygen diphosphate linkages, and it is possible to distinguish between allylic and homoallylic isomers from the intensity of the fragment at *m/z* 177. Peaks due to additional fragmentation of the oxygen–phosphorus bonds were also seen especially for the sodium salts, which may serve as a useful basis for comparison. At this time, we cannot be completely certain whether the phosphate fragment peaks originate from gas phase decomposition of molecular ions or result from bombardment-induced decomposition in the matrix. Our data show that FABMS can be used to detect isoprenoid diphosphates at the microgram level. The technique should be useful for analysis of this class of molecules in biosynthetic and enzyme mechanism studies.

ACKNOWLEDGMENTS

This project was supported by Grants GM-21328 and GM-25521 from the Institute for General Medical Sciences of the National Institutes of Health. Purchase of the mass spectrometer was supported by Grants CHE-8310031 and CHE-8100424 from the National Science Foundation. We thank Dr. Todd Capson for samples of **8**, **15**, and **16**; Dr. Larry Perez for **14**; and Kay Stremler for **9** and **10**.

REFERENCES

1. Spurgeon, S. L., and Porter, J. W. (1981) *in* Biosynthesis of Isoprenoid Compounds (Spurgeon, S. L., and Porter, J. W., Eds.), Vol. 1, pp. 2–46, Wiley Interscience, New York.
2. AkiJokis, E. D., Klee, H., Amasino, R. M., Nester, E. W., and Gordon, M. P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5994–5998.
3. Tidd, B. K. (1971) *J. Chem. Soc. B,* 1168–1176.
4. Poulter, C. D., Muscio, O. J., and Goodfellow, R. J. (1974) *Biochemistry* **13,** 1530–1538.
5. Yang, T.-H., Zabriskie, M. T., and Poulter, C. D. (1984) *J. Chromatogr.* **312,** 121–132.
6. Davisson, V. J., Woodside, A. B., Neal, T. R., Stremler, K. E., Muehlbacher, M., and Poulter, C. D. (1986) *J. Org. Chem.* **51,** 4768–4779.
7. Macfarlane, R. D. (1982) *Acc. Chem. Res.* **15,** 268–275.
8. Barber, M., Bordoli, R. S., Sedgwick, A. N., and Tyler, A. N. (1981) *J. Chem. Soc. Chem. Commun.,* 325–326.
9. Barber, M., Bordoli, R. S., Sedgwick, R. D., and Tyler, A. N. (1981) *Nature (London)* **293,** 270–275.
10. Grotjahn, L., Frank, R., and Blocher, H. (1982) *Nucleic Acids Res.* **293,** 4671–4678.
11. Eagles, J., Javanand, C., and Self, R. (1984) *Biomed. Mass Spectrom.* **11,** 41–46.
12. Aubagnac, J. L., Devienne, F. M., Combarieu, R., Barascut, J. L., Imbach, J. L., and Lazrek, H. B. (1983) *Org. Mass Spectrom.* **18,** 361–364.
13. Ulrich, J., Guy, A., Molko, D., and Teouk, R. (1984) *Org. Mass Spectrom.* **19,** 585–588.
14. Brewer, G., and Grimshaw, C. M. (1984) *Biomed. Mass Spectrom.* **11,** 400–402.
15. Grotjahn, L., Frank, R., and Heisterberg-Montsis, G. (1984) *Tetrahedron Lett.* **25,** 5373–5376.
16. Hogg, A. H., Kelland, J. G., Vederas, J. C., and Tamm, C. (1986) *Helv. Chim. Acta* **69,** 908–917.

17. Hutchinson, D. W., and Sample, G. (1985) *Org. Mass Spectrom.* **20,** 143–145.
18. Burlingame, A. L., Baillie, T. A., and Derrick, P. J. (1986) *Anal. Chem.* **58,** 165R–211R.
19. Kingston, E. E., Benyon, J. H., and Newton, R. P. (1984) *Biomed. Mass Spectrom.* **11,** 367–374.
20. Sherman, W. R., Ackerman, K. E., Berger, R. A., Gish, B. G., and Zinbo, M. (1986) *Biomed. Mass Spectrom.* **13,** 333–341.
21. Connolly, B. A., Eckstein, F., and Grotjahn, L. (1984) *Biochemistry* **23,** 2026–2031.
22. Hilscher, L. W., Hanson, C. D., Russell, D. H., and Rauschel, F. M. (1985) *Biochemistry* **24,** 5888–5893.
23. Clifford, A. J., Silverman-Jones, C. S., Creek, K. E., DeLuca, L. M., and Tondeur, Y. (1985) *Biomed. Mass Spectrom.* **12,** 221–227.
24. Sharp, T. R., Davisson, V. J., and Poulter, C. D. (1985) Paper presented in part at the 33rd Annual Conf. Mass Spectrom. Allied Topics, San Diego, CA 26–31 May.
25. Epstein, W. W., and Rilling, H. C. (1970) *J. Biol. Chem.* **245,** 4597–4605.
26. Capson, T. L. (1986) Ph.D. dissertation, University of Utah, Salt Lake City.
27. Davisson, V. J., Neal, T. R., and Poulter, C. D. (1985) *J. Amer. Chem. Soc.* **107,** 5277–5279.
28. Sharp, T. R. (1986) *Org. Mass Spectrom.* **21,** 793–795.
29. Fujii, I., Isobe, R., and Kanematsu, K. (1985) *J. Chem. Soc. Chem. Commun.,* 405–406.
30. Grover, J. L. (1985) *Biomed. Mass Spectrom.* **12,** 191–196.
31. Verma, S., Pomerantz, S. C., Sethi, S. K., Smith, D. L., and McClosky, J. A. (1986) *Anal. Chem.* **58,** 2898–2902.
32. Neal, T. R. (1981) Ph.D. dissertation, University of Utah, Salt Lake City.
33. Sanders, C. G., Sharp, T. R., and Allred, E. L. (1986) *Tetrahedron Lett.* **27,** 3231–3234.
34. Sharp, T. R., Smith, D. E., Heah, P. C., Bahro, W. E., Crocco, G. L., Bodner, G. S., Fernandez, J. M., and Gladysz, J. A. (1985) *Adv. Mass Spectrom.* **10,** 1359–1360.
35. Sharp, T. R., and Jones, H. K. (1986) Paper presented at 34th Ann. Conf. Mass Spectrom. Allied Topics, Cincinnati, OH.
36. Blackburn, G. M., Englund, D. A., and Kolkman, F. (1981) *J. Chem. Soc. Chem. Commun.,* 930–932.