Amino Acids (2005) 28: 369–372
DOI 10.1007/s00726-005-0207-8

Amino Acids
Printed in Austria

Synthesis, characteristics and biological activity of pentacoordinated spirophosphoranes derived from amino acids

L. Yu, Z. Liu, H. Fang, Q. L. Zeng, and **Y. F. Zhao**

Department of Chemistry, The Key Laboratory for Chemical Biology of Fujian Province, Xiamen University, Xiamen, China

Received March 20, 2005
Accepted March 31, 2005
Published online May 17, 2005;

Summary. The reactions of phosphorus trichloride with various amino acids afford the pentacoordinated spirophosphoranes. The reaction procedures were traced by ^{31}P NMR spectra techniques. A new crystal structure of alanine derivative was characterized, which is a slightly distorted TBP structure. Besides, this kind of spirophosphoranes are potent inhibitors to tyrosinase.

Keywords: Pentacoordinated spirophosphoranes – ^{31}P NMR – TBP – Tyrosinase inhibition

Introduction

Phosphorus element was first discovered in organism in the 17th century. After half-century's study, it was found that phosphorus element played a crucial role in many aspects of bio-system. For example, protein phosphorylation and dephosphorylation has long been an important field in biochemistry research (Ao, 1994). The pentacoordinate phosphorus structure is proposed as the key structure to study this problem. Since 1960s, much attention has been paid to pentacovalent phosphorus compounds in organophosphorus chemistry (Ramirez, 1968). We have done a lot of research in this field (Zhao and Zhao, 1998; Fu et al., 1997, 1999; Li et al., 1992; Ma et al., 1992; Zhao et al., 1993). Spirophosphoranes derived from amino acids were seldom studied. To our best knowledge, only Munoz group reported the preparation of symmetric spirophosphoranes derived from 2-aminoisobutyric acid and L-alanine (Garrigues et al., 1977) but their biological activity has not been investigated. In our previous communication, the fragmentation characteristics of the new kind of pentacoordinated spirophosphoranes derived from amino acids in electrospray ionization mass spectra (ESI-MS) was reported (Liu et al., 2003). Here we report the preparation, characteristics and bioactivity of this new series of pentacoordinated spirophosphoranes, and structure analysis by single crystal X-ray diffraction.

Material and methods

The solvent and reagent were dried and purified by standard method before use. Triethylamine and glycol dimethyl ether were refluxed and then distilled over sodium. ^{1}H NMR spectra were recorded at 500 MHz using $CDCl_3$ as solvent. ^{31}P NMR spectra were determined at 202.4 MHz using $CDCl_3$ as solvent, and positive chemical shifts are downfield from 85% H_3PO_4 used as an external reference. High Resolution ESI-MS data were obtained using APEX II FT-ICRMS, while the ESI-SI ones were determined with a Bruker ESQYIRE ~ 3000 plus. Operating conditions for ESI in the positive ion mode were as follows: spray voltage, 4000 V, target, 207 m/z, capillary temperature, 300°C; dry gas (N_2), 10 L/min, Nebulizer (N_2), 30 psi. Mass spectra were registered in the scan range from 50 to 350 m/z. X-ray measurements were carried out on Bruker APEX area-detector diractometer.

As a general procedure, phosphorus trichloride (10 mmol) was added to a stirred amino acid (20 mmol) in glycol dimethyl ether under a nitrogen atmosphere at R.T., and then triethylamine (30 mmol) was added dropwise to the solution at −10°C to induce the reaction. After completion of the addition, the solution was stirred for two more hours. The solvent was removed under reduced pressure by rotary evaporation and the residue was washed three times with water rapidly and adequately. The crude product was filtered and recrystallized from acetone and petroleum ether (1:1). Yields and the physical data of the pentacoordinated spirophosphorane investigated are given as following:

Compound **4a** Yield 1.08 g (52%), mp 180.0 ~ 181.0°C. ^{31}P NMR ($CDCl_3$): δ −68.10. ^{1}H NMR ($CDCl_3$): δ 1.44 (d, 6H, C**H**$_3$), 3.96 (m, 2H, C**H**), 3.47 (br, 2H, N**H**), 7.46 (d, J = 815 MHz, 1H, P**H**). ^{13}C NMR: δ 171.69 (s, **C**=O), 49.61 (**C**H), 49.57 (**C**H), 20.19 (s, **C**H$_3$). High-Resolution ESI-MS m/z: 207.0528 $[M+H]^+$. Calculated 207.0490.

Compound **4b** Yield 1.65 g (62.6%), mp 181.0 ~ 182.0°C. ^{31}P NMR ($CDCl_3$): δ −67.25 (64%), −70.27 (36%). ^{1}H NMR: δ 0.95 (d, 6H, C**H**$_3$), 0.98 (d, 6H, C**H**$_3$), 2.19 (m, 2H, C**H**(CH$_3$)$_2$), 3.54 (m, 2H, CHN**H**), 3.82

(m, 2H, NHC**H**), 7.38, 7.42 (m, 1H, $^1J = 819$ Hz P–**H**). ESI-MS *m/z*: 263 $[M+H]^+$.

Compound **4c** Yield 1.84 g (63.4%), mp 186.0 ~ 187.0°C. ^{31}P NMR ($CDCl_3$): δ −67.49 (60%), δ −69.97 (40%). ^{1}H NMR: δ 0.94 (d, 6H, CH_3), 0.98 (d, 6H, CH_3), 1.75 (d, 4H, $CH_2CH(CH_3)_2$), 1.51 (m, 2H, $CH(CH_3)_2$), 3.53 (m, 2H, CHN**H**), 3.89 (m, 2H, NHC**H**), 7.34, 7.41 (m, 1H, P–H). ESI-MS m/z: 291 $[M+H]^+$.

Compound **4d** Yield 2.34 g (80.3%), mp 185.5 ~ 187.0°C. ^{31}P NMR ($CDCl_3$): δ −67.65 (67%), −70.49 (33%). ^{1}H NMR: δ 0.93 (d, 6H, CH_2CH_3), 1.00 (d, 6H, $CHCH_3$), 1.37 (d, 4H, CH_2CH_3), 1.87 (m, 2H, $CHCH_3$), 3.54 (m, 2H, CHN**H**), 3.85 (m, 2H, NHC**H**), 7.41, 7.34 (m, 1H, P–H). ESI-MS m/z: 291 $[M+H]^+$.

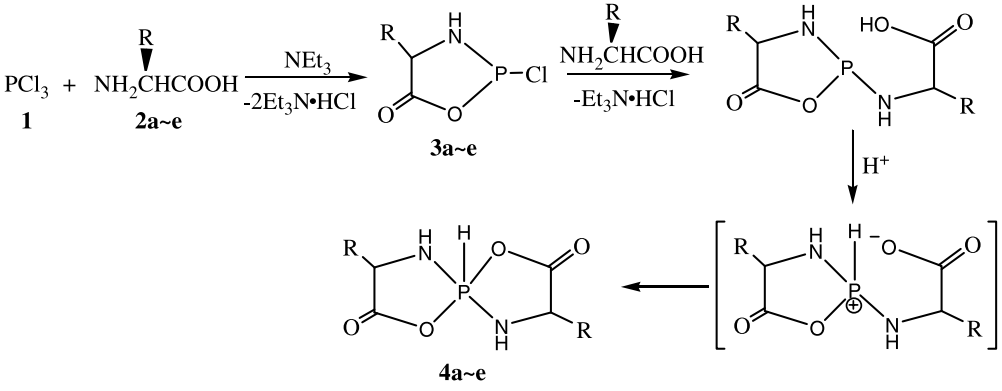
Compound **4e** Yield 2.38 g, (66.3%), mp 224.0 ~ 225.0°C. ^{31}P NMR ($CDCl_3$): δ −66.80 (55%), −69.17 (45%). ^{1}H NMR: δ 3.15 (d, 2H, CHaHb–Ph), 3.21 (d, 2H, CHaHb–Ph), 3.30 (br, 2H, NHCH), 4.053 (d, 2H, NH), 7.10 ~ 7.33 (m, 10H, Ph-H), 7.26, 7.38 (m, 1H, P–H). ESI-MS m/z: 359 $[M+H]^+$.

The biochemical activity assay was performed as following (Kubo et al., 2000): pure samples of spirophosphoranes were dissolved in DMSO respectively as 10 mg in 1 ml solution. First, 0.05 mM phosphate buffer (pH 6.8) was incubated at 30°C for 10 min. Then, 0.3 ml of L-DOPA solution, 0.1 ml of the sample solution and 0.1 ml of the aqueous solution of mushroom tyrosinase were added to immediately, the initial rate was measured in optical density at 475 nm on the basis of the formation of dopachrome. Results of activity for compounds **4a–d** are summarized in Table 3. While testing compound **4e**, a precipitate was formed and the test could not be completed.

Results and discussion

In the present work, we synthesized a series of spirophosphoranes, namely, 3,8-Dialkyl-1,6-dioxa-4,9-diaza-5λ^5-phospha-spiro [4.4] nonane-2,7-dione in IUPAC name. These pentacoordinated spirophosphoranes derived from amino acids (Scheme 1) are easily obtained with reasonable yields from 52% ~ 80.3% (Table 1). This kind of compounds are stable with high melting point around 180 ~ 225°C.

The ^{31}P nucleus is a very sensitive probe for structural studies of organophosphorus compounds. Therefore, the reaction was traced by ^{31}P NMR spectroscopy. For example (Fig. 1), reagent **1** stirred with alanine in glycol dimethyl ether shows a signal in the ^{31}P NMR spectrum at 220 ppm, then with the addition of triethyl amine, a new ^{31}P NMR signal at 164 ppm appeared, which indicated the formation of the tricoordinated phosphorus substances (**3a**). As the reaction continued, the inter-mediate gradually decreased and converted into **4a**, which exhibited a doublet peak due to ^{31}P coupled with ^{1}H at the neighborhood of −68 ppm. By the ^{31}P NMR spectrum analysis, it seemed that the conversion is almost quantitative. The mechanism of the reaction was proposed in Scheme 1. The process of other reactions were similar, but the final



2a~e stand for alanine, valine, leucine, isoleucine, phenylalanine respectively

Scheme 1. Synthesis and possible mechanism of pentacoordinated spirophosphoranes

Table 1. Physical dates of compounds **4a ~ e**

Compound	R	mp (°C)	Yield (%)	^{31}P NMR ($CDCl_3$) δ (ppm)	ESI-MS (m/z) $[M+H]^+$
4a	$-CH_3$	180.0 ~ 181.0	52	−68.10	207
4b	$-CH(CH_3)_2$	181.0 ~ 182.0	62.6	−67.25, −70.27	263
4c	$-CH_2CH(CH_3)_2$	186.0 ~ 187.0	63.4	−67.49, −69.97	291
4d	$-CH(CH_2CH_3)CH_3$	185.5 ~ 187.0	80.3	−67.65, −70.49	291
4e	$-CH_2Ph$	224.0 ~ 225.0	66.3	−66.80, −69.17	359

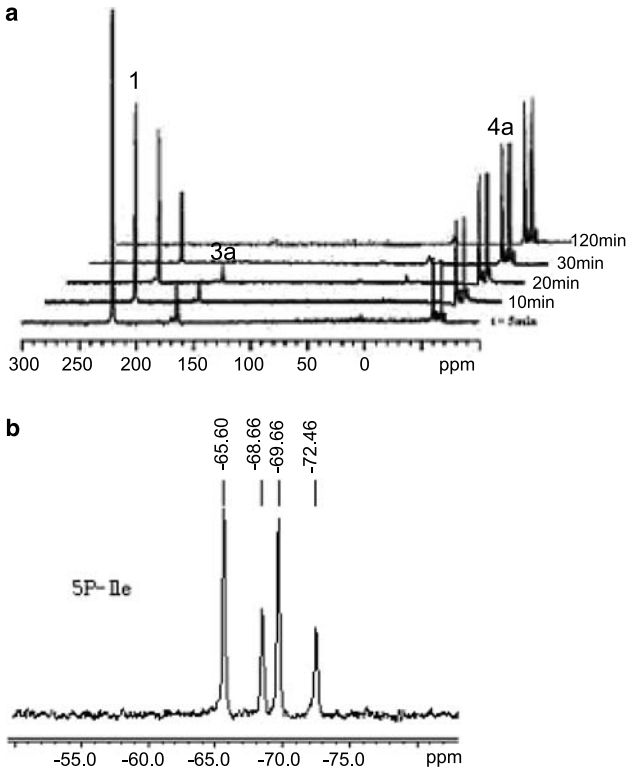


Fig. 1. **a** The ^{31}P NMR stacked spectra for synthesis of compound **4a** (undecoupled). **b** The ^{31}P NMR spectra for compound **4d** (undecoupled)

products **4b** $\sim$ **e** exhibited different chemical shifts in ^{31}P NMR spectrums. There was a pair of doublet peaks at high field (Fig. 1), which indicated that a pair of diastereoisomeric products were formed. All of them showed ^{31}P NMR spectra in the range of -65 to about -75 ppm with a P–H coupling constant about 820 Hz.

The compound **4a**, 3,8-Dimethyl-1,6-dioxa-4,9-diaza-5λ^5-phospha-spiro [4.4] nonane-2,7-dione, was cultured for the first time as a colorless chunk crystal. The crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number: CCDC 260461. The ORTEP plot of the molecular structure for **4a** is shown in Fig. 2 with atomic labeling scheme while the selected bond lengths, bond angles, and torsion angles are shown in Table 2.

X-ray structural analysis of **4a** revealed that the phosphorus atom configuration in the compound was distorted trigonal bipyramid (TBP) with the two oxygen atoms in apical position and two nitrogen atoms in equatorial position, as showed in Fig. 2. The N1–P–N1# angle 127.7° is larger than 120° for an ideal TBP. The five-member ring O1#–P–N1# angles of 89.0° and the Interring O1–P–N1# angles of 90.4° showed insignificant deviations from an ideal angle 90°. The O1–P–O1# angle is 2.1° smaller than

Table 2. Selected bond lengths (Å) and angles (°) for **4a**

P(1A)–N(1A)#1	1.627(6)	N(1A)#1–P(1A)–N(1A)	127.7(5)
P(1A)–N(1A)	1.627(6)	N(1A)#1–P(1A)–O(1A)	90.4(3)
P(1A)–O(1A)	1.736(5)	N(1A)–P(1A)–O(1A)	89.0(3)
P(1A)–O(1A)#1	1.736(5)	N(1A)#1–P(1A)–O(1A)#1	89.0(3)
P(1A)–H(1)	1.43(8)	N(1A)–P(1A)–O(1A)#1	90.4(3)
O(1A)–C(1A)	1.317(10)	O(1A)–P(1A)–O(1A)#1	177.9(5)
O(1A)–C(1A)	1.198(10)	N(1A)#1–P(1A)–H(1)	116.1(3)
N(1A)–C(3A)	1.456(10)	N(1A)–P(1A)–H(1)	116.1(3)
N(1A)–H(1AA)	0.8600	O(2A)–P(1A)–H(1)	91.0(3)
C(3A)–C(2A)	1.512(11)	O(2A)#1–P(1A)–H(1)	91.0(2)
C(3A)–C(1A)	1.517(11)	C(1A)–O(2A)–P(1A)	116.5(5)
C(3A)–H(3AA)	0.9800	C(3A)–N(1A)–P(1A)	119.0(5)
C(2A)–H(2AA)	0.9600		
C(2A)–H(2AB)	0.9600		
C(2A)–H(2AC)	0.9600		

the ideal 180° (Newton et al., 1974; Sheldrick, 1997; Narayanan et al., 1976). Two intermolecular hydrogen bonds formed in the crystal, as depicted in Fig. 3. N–H is the donor to O, with D–A distance of 3.01Å and D–H...A angle of 155°. This ring of two hydrogen bonds links molecules along the *a* axis.

The spirophosphoranes were tested for their inhibitory activity to tyrosinase, an important enzyme in the formation of melanin widely distributed in microorganisms,

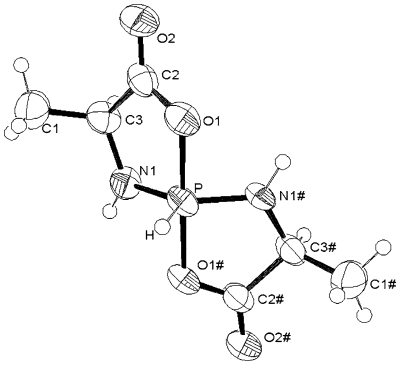


Fig. 2. ORTEP view of **4a** showing the thermal ellipsoids at the 50% probability level. Symmetry transformations used to generate equivalent atoms: # $-x+2$, y, $-z+1/2$

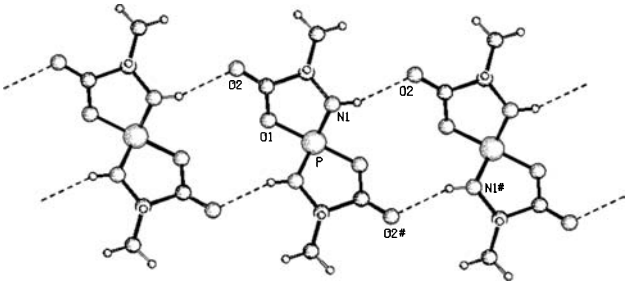


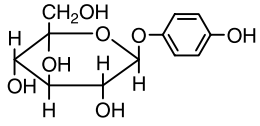
Fig. 3. Hydrogen bonds (dashed lines) in the crystal structure, viewed along the *a* axis

Table 3. Rate of inhibition to tyrosinase of the compounds (**4a** ∼ **d**)

Compounds	Arbutin[22]	**4a**	**4b**	**4c**	**4d**
Rate of inhibition (%)	88	63.5	45	73	96

animals and plants (Seo et al., 2003). The tyrosinase is the key enzyme in melanin biosynthesis (Shiino et al., 2002) which catalyzes the hydroxylation of L-tyrosine to 3,4-dihydroxy-L-phenylalanine and its subsequent oxidation to dopaquinone. Therefore, inhibitors of tyrosinase should be useful as therapeutic agents for the treatment of melanin hyperpigmentation and cosmetic materials for whitening (Maeda, 1996). Moreover, tyrosinase is responsible for the undesired enzymatic browning of fruits and vegetables (Martinez and Whitaker, 1995) that takes place during the course of postharvest handling. Thus, in the food industry, tyrosinase inhibitors are used to prevent enzymatic browning (Kubo et al., 2000a, b).

According to the ratio of initial activity to the remaining, we got the inhibition rates of samples towards tyrosinase. The result is summarized in Table 3. It is noticeable that these spirophosphoranes have tyrosinase inhibition activity, especially the compound containing Ileucine residue shows 96% rate of inhibition, which is better than the cosmetic whitening reagent Arbutin (Liu et al., 2000).



Arbutin

Conclusion

In summary, a series of new spirophosphoranes derived from amino acids were obtained. The reaction process was traced by ^{31}P NMR, and thus a mechanism was proposed. A single crystal of spirophosphoranes **4a** was cultured for the first time, and its X-ray structure was elucidated. Besides, these compounds had a distinct inhibition activity of tyrosinase. Especially the compound **4d** shows 96% rate of inhibition, which is better than Arbutin.

Acknowledgements

The authors would like to thank the Fujian Foundation of Science and Technology (No. 2001F008), Fujian Key Foundation of Science and Technology (No. 2002H011) for supporting this work.

References

Ao SZ (1994) Cell regulation by protein phosphorylation and dephosphorylation. Academic Shang Hai Sci Tech

Fu H, Tu GZ, Li ZL, Zhao YF, Zhang RQ (1997) New and efficient approach to the synthesis of pentacoordinate spirobicyclic phosphoranes. J Chem Soc Perkin Trans 1: 2021–2022

Fu H, Li ZL, Zhao YF, Tu GZ (1999) Oligomerization of *N*,*O*-Bis(trimethylsilyl)-α-amino acids into peptides mediated by *o*-phenylene phosphorochloridate. J Am Chem Soc 121: 291–295

Garrigues B, Munoz A, Koenig M, Sanchez M, Wolf R (1977) Spirophosphoranylation d'alpha-aminoacides-I – Preparation et tautomerie. Tetrahedron 33: 635

Kubo I, Ikuyo KH, Chaudhuri SK, Kubo Y, Sanchez Y, Ogura T (2000a) Flavonols from Heterotheca inuloides: Tyrosinase inhibitory activity and structural criteria. Bioorgan Med Chem 8: 1749–1755

Kubo I, Ikuyo KH, Kubo Y, Yamagiwa Y, Kamikawa T, Haraguchi H (2000b) Molecular design of antibrowning agents. J Agric Food Chem 48: 1393–1399

Li YM, Yin YW, Zhao YF (1992) Phosphoryl group participation leads to peptide formation from N-phosphorylamino acids. J Peptide Protein Res 39: 375

Liu YH, Li CG, Peng JL (2000) Study of chemical evaluation of the activity of skin lighteners. Fine Chemicals 17: 318

Liu Z, Yu L, Chen Y, Zhou N, Chen J, Zhu CJ, Xin B, Zhao YF (2003) Interesting differences between the protonated and sodium adducts of pentacoordinated bisaminoacylspirophosphoranes in electrospray ionization mass. Spectrometry J Mass Spectrom 38: 231–233

Ma XB, Zhao YF (1992) The synthesis and Novel properties of N-phosphoryl peptides. Phosphorus Sulfur Silicon Relat Elements 66: 107

Maeda K, Fukuda M (1996) Arbutin: mechanism of its depigmenting action in human melanocyte culture. J Pharmacol Exp Ther 276: 765–769

Martinez MV, Whitaker JR (1995) The biochemistry and control of enzymatic browning. Trends Food Sci Technol 6: 195–200

Newton MG, Collier JE, Wolf R (1974) Crystal and molecular structure of a dioxadiazaspirophosphorane derived from (−)-ephedrine. J Am Chem Soc 96: 6888–6893

Narayanan P, Berman HM, Ramirez F, Marecek JF, Chaw YF, Prasad VAV (1976) Crystal and molecular structure of oxyphosphoranes. 9.[1] The spiropentaoxyphosphorane, (PO_5) ($CF_3C{=}CCF_3$) (C_6H_4) (C_6H_5). J Am Chem Soc 99: 3336–3342

Ramirez F (1968) Oxyphosphoranes. Acc Chem Res 1: 168

Seo SY, Sharma VK, Sharma N (2003) Mushroom tyrosinase: recent prospects. J Agric Food Chem 51: 2837–2853

Sheldrick GM (1997) SHELEX97, program package for crystal structure solution and refinement. University of Göttingen, Germany

Shiino M, Watanabe Y, Umezawab K (2002) Synthesis and tyrosinase inhibitory activity of novel N-hydroxybenzyl-N-nitrosohydroxylamines. Bioorg Chem 31: 129–135

Zhao YF, Zhao GH (1998) Elementary organic chemistry. Academic, Tsing Hua University

Zhao YF, Li YM, Yin YW, Li YC (1993) The regulation effect of phosphoryl group on amino acid side chain. Sci in China 36: 1451

Authors' address: Prof. Yufen Zhao and Dr. Qingle Zeng, Department of Chemistry, The Key Laboratory for Chemical Biology of Fujian Province, Xiamen University, Xiamen 361005, China,
Fax: +86-2185780, E-mail: yfzhao@xmu.edu.cn, qinglezeng@yahoo.com