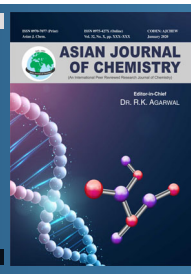
Asian Journal of Chemistry; Vol. 32, No. 2 (2020), 329-334

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22381



Pyridine Alkaloid Derivatives of Dithiophosphoric Acids and Their Antimicrobial Evaluation

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Received: 5 August 2019; *Accepted*: 24 September 2019; *Published online*: 30 December 2019; AJC-19721

A new series of (*S*)-(–)-nicotinium salts of cyclic dithiophosphoric acids were synthesized by the reactions of 5,5-dimethyl-2-mercapto-2-thiono-1,3,2-dioxaphosphorinane and 4-methyl-2-mercapto-2-thiono-1,3,2-dioxaphosphorinane with (*S*)-(–)-nicotine. Picolinic and nicotinic acids and potassium pyridine-3-carboxylate reacted with *O,O*-diterpenyl dithiophosphoric acids on the basis of (1*R*)-*endo*-(+)-fenchyl alcohol and (*S*)-(–)-menthol to afford the corresponding 2-carboxypyridinium, 3-carboxypyridinium and potassium pyridinium-3-carboxylate dithiophosphates. The antibacterial and antifungal activity of (*S*)-(–)-nicotinium 4-methyl-2-mercapto-2-thiono-1,3,2-dioxaphosphorinane and salts of *O,O*-diterpenyl dithiophosphoric acids on the basis of nicotine and nicotinamide were evaluated.

Keywords: Nicotine, Nicotinic acid, Picolinic acid, Dithiophosphoric acids, Antimicrobial.

INTRODUCTION

Alkaloids attract a great attention as a scaffold for generation of bioactive derivatives [1]. Among them, pyridine alkaloids based on a pyridine cycle such as vitamin B_3 (nicotinic acid and nicotinamide) and nicotine are the most promising structures [2]. Vitamin B_3 is water soluble cofactor for enzymatic reactions in bacterial cells [3]. Isonicotinic acid hydrazide (isoniazid) derivatives, 2-benzylsulfanyl-nicotinic acid based on 1,3,4-oxadiazoles, metal complexes of Schiff-bases derived from 2-aminonicotinic acid and salicylaldehyde, 2-amino-5-arylazo-6-aryl substituted nicotinic acid and pyrido[2,3-d]-pyrimidine and N-acylhydrazones of nicotinic acid hydrazides exhibited antibacterial activity [4-7]. Such pyridine derivative as [3-(5-(3-fluorophenyl)nicotinoyl)-1-methylpyrrolidin-2-one] showed the lowest minimal inhibitory concentrations (MIC) value against *Mycobacterium tuberculosis* of 1 μg/mL [8]. 2-Thio- and N-substituted nicotinamide derivatives are active against phytopathogenic fungi [9]. The trigonelline derivative of 1-methyl-N'-(hydroxymethyl)nicotinamide chloride possesses antibacterial and inflammatory activity [10].

It should be noted that the most of the pyridine alkaloid derivatives possess covalent structures. Much less attention has been paid to ionic structures based on bioactive pyridine alkaloid derivatives. Thus, sodium salt of nicotinic acid inhibited cholesterol atherosclerosis [11]. Meanwhile, ionic-liquids on the basis of salts of dithiophosphoric acids can exhibit high physiological activity. Thus, have recently found that glutathione salts of dithiophosphoric acids possess redox-modulating and antiproliferative effects on cancer cells [12]. In addition, other pyridine alkaloids such as pyridoxine, nicotine and nicotinamide and cinchona alkaloids give corresponding salts of dithiophosphoric and aryldithiophosphonic acids possessed antimicrobial activity [13,14]. We have now extended our approach to nicotine derivatives of cyclic dithiophosphoric acids to evaluate their antimicrobial activity. The antibacterial and antifungal activity of previously obtained salts of *O,O*-diterpenyl dithiophosphoric acids on the basis of nicotine and nicotinamide [13] were also summarized in this paper. In this study, nicotinic acids and its isomers and potassium derivative were also involved in the reactions with dithiophosphoric acids

on the basis of (1*R*)-*endo*-(+)-fenchyl alcohol and (*S*)-(–)-menthol.

EXPERIMENTAL

The reactions were carried out under a flow of dry argon. Benzene and ethanol were dried prior to use. Tetraphosphorus decasulfide (purity 99 %), (*S*)-(–)-nicotine (purity 99 %), picolinic acid (purity 99 %), nicotinic acid (purity 98 %) and (1*R*)-*endo*-(+)-fenchyl alcohol (purity 96 %) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Potassium pyridine-3-carboxylate (purity 98 %) was purchased from Molbase Chemical (Shanghai, China). 2,2-Dimethyl 1,3-propanediol (purity 98 %) was purchased from Fluka kemika. Butane-1,3-diol (purity 99 %) was purchased from Merck (Kenilworth, NJ, USA). (*R*)-(+)-Menthol (purity 99 %) was purchased from Alfa Aesar (Heysham, UK). 5,5-Dimethyl-2-mercapto-2-thiono-1,3,2-dioxaphosphorinane (**1a**) and 4-methyl-2-mercapto-2-thiono-1,3,2-dioxaphosphorinane (**1b**) were obtained according to published procedure [15]. *O,O*-Di[(–)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohex-1-yl]dithiophosphoric acid (**4a**) was synthesized by the reactions of tetraphosphorus decasulfide with (*S*)-(–)-menthol as reported recently [15]. *O,O*-Di[(1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl) dithiophosphoric acid (**4b**) was prepared by the reaction of tetraphosphorus decasulfide with (1*R*)-*endo*-(+)-fenchyl alcohol [16].

Synthesis of 3a: (*S*)-(–)-Nicotine (**2**) (0.24 g, 1.5 mmol) was added portionwise under dry argon with stirring at 20 °C to the solution of acid **1a** (0.3 g, 1.5 mmol) in 10 mL of anhydrous benzene. The mixture was heated for 1.5 h at 50 °C. The resulting clear solution was evaporated at reduced pressure (0.5 mm Hg) at 40 °C for 1 h and then in vacuum (0.02 mm Hg) for 1 h to give (*S*)-(–)-nicotinium 5,5-dimethyl-2-mercapto-2-thiono-1,3,2-dioxaphosphorinane (**3a**) (0.42 g, 78 %). FTIR (film, ν_{max}, cm^{-1}): 3089 w, 3030 w (=C-H_{Ar}), 2958 m, 2872 m [$\nu_{as,s}(CH_3, CH_2)$], 2687 m (NH^+), 1642 w, 1595 w, 1579 w (C=C_{Ar}), 1471 s, 1434 s [$\delta_{as}(CH_3)$], 1397 m, 1367 m {$\delta_s[(CH_3)_2C_{gem.}]$}, 1051 *vs*. [(P)O–C], 997 *vs*. (O–C, OC–C), 697 *vs*. (P=S), 546 m (P–S). ^{1}H NMR (CD_3OD): δ = 1.06 s [6H, ($C^{7,8}H_3$)$_2$C], 2.36 m (2H, $C^{4'}H_2$, 2H, $C^{3'}H_2$), 2.50 m (2H, $C^{2'}H_2$), 2.76 s (3H, $NC^{6'}H_3$), 3.36 d (1H, $C^{5'}H$, $^3J_{HH}$ = 5.6 Hz), 3.97 d (2H, C^4H_2OP, 2H, C^6H_2OP, $^3J_{PH}$ = 15.4 Hz), 7.54 d (1H, $C^{11'}H$, $^3J_{HH}$ = 8.0 Hz), 7.55 d (1H, $C^{11'}H$, $^3J_{HH}$ = 8.0 Hz), 8.22 d (1H, $C^{12'}H$, $^3J_{HH}$ = 6.3 Hz), 8.62 m (1H, $C^{10'}H$), 8.71 s (1H, $C^{8'}H$) ppm. $^{31}P\{^1H\}$ NMR (benzene): δ = 108.7 ppm. Elemental analysis for $C_{15}H_{25}N_2O_2PS_2$ calculated (%): C, 49.98; H, 6.99; N, 7.77; P, 8.59; S, 17.79; found (%): C, 49.65; H, 6.67; N, 7.45; P, 8.88; S, 18.12.

(*S*)-(–)-Nicotinium 4-methyl-2-mercapto-2-thiono-1,3,2-dioxaphosphorinane (**3b**) was obtained similarly. Yield 77 %. FTIR (film, ν_{max}, cm^{-1}): 3014 w (=C-H_{Ar}), 2971 s, 2884 m [$\nu_{as,s}(CH_3, CH_2)$], 2690 m (NH^+), 1656 w, 1595 w, 1579 w (C=C_{Ar}), 1455 m, 1434 *vs*. [$\delta_{as}(CH_3)$], 1384 m [$\delta_s(CH_3)$], 1028 *vs*. [(P)O–C], 958 *vs*. (O–C, OC–C), 696 *vs*. (P=S), 530 m (P–S). ^{1}H NMR (CD_3OD): δ = 1.248 d (3H, C^7H_3, $^3J_{HH}$ = 7.2 Hz), 1.254 d (3H, C^7H_3, $^3J_{HH}$ = 7.2 Hz), 1.65 m (2H, C^5H_2), 1.81 m (C^6H_2), 2.31 m (2H, $C^{3'}H_2$), 2.38 m (2H, $C^{4'}H_2$), 2.58 m (2H, $C^{2'}H_2$), 2.77 s (3H, $NC^{6'}H_3$), 3.33 m (1H, $C^{5'}H$), 4.61 m (C^4H), 7.57 d (1H, $C^{11'}H$, $^3J_{HH}$ = 7.7 Hz), 7.58 d (1H, $C^{11'}H$, $^3J_{HH}$ = 7.7 Hz), 8.154 d (1H, $C^{12'}H$, $^3J_{HH}$ = 8.2 Hz), 8.65 m (1H, $C^{10'}H$), 8.750 s (1H, $C^{8'}H$), 8.753 s (1H, $C^{8'}H$) ppm. $^{31}P\{^1H\}$ NMR (benzene): δ = 108.0 ppm. The mass-spectrum of ESI (acetone) *m/e:* 368.4 $[M + Na]^+$ (calculated *M* 346.4). Elemental analysis for $C_{14}H_{23}N_2O_2PS_2$ calculated (%): C, 48.54; H, 6.69; N, 8.09; P, 8.94; S, 18.51; found (%): C, 48.23; H, 6.32; N, 7.78; P, 9.13; S, 18.87.

Synthesis of 6a: The mixture of acid **4a** (1.1 g, 2.7 mmol) and picolinic acid (**5a**) (0.34 g, 2.8 mmol) in 10 mL of anhydrous ethanol was heated for 1.5 h at 60 °C. The resulting clear solution was evaporated at reduced pressure (0.5 mm Hg) at 40 °C for 1 h and then in vacuum (0.02 mm Hg) for 1 h to give 2-carboxypyridinium *O,O*-di[(–)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohex-1-yl] dithiophosphate (**6a**) (0.66 g, 85 %) as white solid, m.p.: 63–65 °C. FTIR (film, ν_{max}, cm^{-1}): 3350 m vbr (H–O), 3079 w, 3030 w (=C-H_{Ar}), 2954 s, 2924 s, 2869 m [$\nu_{as,s}(CH_3, CH_2)$], 2706 w (NH^+), 1708 m (O–C=O), 1603 s, 1526 m (C=C_{Ar}), 1456 m [$\delta_{as}(CH_3)$], 1386 m 1369 m {$\delta_s[(CH_3)_2C_{gem.}]$}, 1023 m [(P)O–C], 974 s, 963 s (O–C, OC–C), 662 m (P=S), 547 m (P–S) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 0.83 d (6H, C<u>H</u>$_3$CH, $^3J_{HH}$ = 8.8 Hz), 0.86 d (6H, C<u>H</u>$_3$CH, $^3J_{HH}$ = 8.8 Hz), 0.94 d (12H, (C<u>H</u>$_3$)$_2$CH, $^3J_{HH}$ = 6.6 Hz), 0.95 d (12H, (C<u>H</u>$_3$)$_2$CH, $^3J_{HH}$ = 6.6 Hz), 1.15 m [2H, (CH$_3$)$_2$C<u>H</u>-CH), 1.43 m (2H, CH$_3$C<u>H</u>), 1.49 s (12H, [C<u>H</u>$_3$)$_2$C], 2.69 s (3H, C<u>H</u>$_3$), 1.69 m (4H, CH$_3$CHC<u>H</u>$_2$CH$_2$), 1.99 d (2H, POCHCH<u>H</u>-*a*, $^3J_{HH}$ = 11.7 Hz), 2.09-2.21 m (4H, POCHCHC<u>H</u>$_2$), 2.38 d (2H, POCHCH<u>H</u>-*e*, $^3J_{HH}$ = 11.7 Hz), 3.44 ddt (2H, POCHC<u>H</u>, $^3J_{HH}$ = 6.6 Hz), 4.46 ddt (2H, POCH, $^3J_{HH}$ = 6.6 Hz, $^3J_{PH}$ = 11.0 Hz), 7.59 m (1H, $C^{5'}H$), 7.96 m (1H, $C^{4'}H$), 8.50 m (1H, $C^{3'}H$), 8.73 m (1H, $C^{6'}H$) ppm. ^{13}C NMR (acetone-d_6, in parentheses is a view of signal in ^{13}C-{^{1}H} NMR): δ = 16.1 q (s) (C^9H_3, $^1J_{CH}$ = 119.6 Hz), 21.0 q (s) ($C^{10}H_3$, $^1J_{CH}$ = 125.4 Hz), 22.0 q (s) (C^8H_3, $^1J_{CH}$ = 124.0 Hz), 22.2 q (s) (C^8H_3, $^1J_{CH}$ = 124.0 Hz), 22.9 t (s) (C^3H_2, $^1J_{CH}$ = 146.0 Hz), 23.1 t (s) (C^3H_2, $^1J_{CH}$ = 146.0 Hz), 25.5 d (s) (C^7H, $^1J_{CH}$ = 126.2 Hz), 25.8 d (s) (C^7H, $^1J_{CH}$ = 126.2 Hz), 31.56 d (s) (C^5H, $^1J_{CH}$ = 120.3 Hz), 31.63 d (s) (C^5H, $^1J_{CH}$ = 120.3 Hz), 34.0 t (s) (C^4H_2, $^1J_{CH}$ = 124.7 Hz), 34.5 t (s) (C^4H_2, $^1J_{CH}$ = 126.2 Hz), 42.6 t (s) (C^6H_2, $^1J_{CH}$ = 126.9 Hz), 44.9 t (s) (C^6H_2, $^1J_{CH}$ = 126.2 Hz), 50.0 d (s) (C^2H, $^1J_{CH}$ = 121.0 Hz), 81.2 dd (d) (HC^{2}OP, $^2J_{CP}$ = 8.4 Hz, $^1J_{CH}$ = 140.1 Hz), 124.5 d (s) ($C^{3'}H$, $^1J_{CH}$ = 166.5 Hz), 127.7 d (s) ($C^{5'}H$, $^1J_{CH}$ = 166.1 Hz), 138.7 d (s) ($C^{4'}H$, $^1J_{CH}$ = 164.3 Hz), 138.7 s (s) ($C^{2'}$), 148.1 d (c) ($C^{6'}H$, $^1J_{CH}$ = 181.9 Hz), 164.9 s (s) ($C^{7'}$=O) ppm. $^{31}P\{^1H\}$ NMR (ethanol): δ = 101.3 ppm. The mass-spectrum of ESI (acetone) *m/e:* 568.6 $[M + K]^+$ (calculated *M* 529.7.). Elemental analysis for $C_{26}H_{44}NO_4PS_2$ calculated (%): C, 58.95; H, 8.37; N, 2.64; P, 5.85; S, 12.11; found (%): C, 59.26; H, 8.43; N, 2.81; P, 6.11; S, 12.47.

3-Carboxypyridinium *O,O*-di[(1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl) dithiophosphate (**6b**) was prepared similarly, however the reaction was carried out at 50 °C. White solid, yield 83 %. FTIR (KBr): ν_{max} = 3430 mbr (H–O), 3079 vw (=C-H_{Ar}), 2957 vs, 2873 s [$\nu_{as,s}(CH_3, CH_2)$], 2726 w (NH^+), 1710 m (O=C–O), 1597 m (C=C_{Ar}), 1461 m [$\delta_{as}(CH_3)$], 1378 m [$\delta_s(CH_3)$], 1003 s, 990 m [(P)O–C], 916 m (O–C, OC–C), 677 m (P=S), 565 m (P–S) ppm. ^{13}C{^{1}H} NMR (acetone-d_6): δ = 20.0 s (C^8H_3), 21.0 s (C^9H_3), 22.1 s ($C^{10}H_3$), 25.8 s (C^5H_2), 26.6 s (C^5H_2), 26.9 s (C^6H_2), 27.0 s (C^6H_2), 31.5

s (C^3H_2), 41.7 s (C^4H), 49.0 s (C^7), 49.1 s (C^1), 84.6 s (POC^2H), 114.3 s ($C^{5'}H$), 126.0 s ($C^{3'}H$), 139.0 s ($C^{4'}H$), 153.3 s ($C^{6'}H$, $C^{2'}H$), 166.0 s ($C^{7'}$=O) ppm. $^{31}P\{^1H\}$ NMR (ethanol): δ = 106.4 ppm. The mass-spectrum of ESI (acetone) *m/e*: 1072.2 [2M + Na]$^+$ (calculated *M* 525.7). Elemental analysis for $C_{26}H_{40}NO_4PS_2$ calculated (%): C, 59.40; H, 7.67; N, 2.66; P, 5.89; S, 12.20; found (%): C, 59.06; H, 7.43; N, 2.91; P, 5.56; S, 12.56.

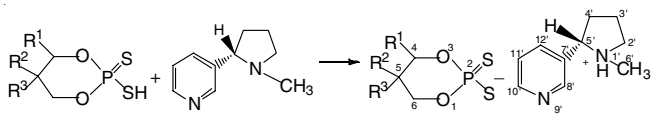
Potassium pyridinium-3-carboxylate *O,O*-di[(1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl) dithiophosphate (**6c**) was prepared similarly, however the reaction was carried out at 20 °C. White solid, yield 80 %, m.p.: 176 °C. FTIR (KBr, ν_{max}, cm^{-1}): 2953 vs, 2708 w [$\nu_{as,s}(CH_3, CH_2)$], 2708 w (NH^+), 1702 m (O=C–O), 1607 m, 1590 m (C=C_{Ar}), 1460 m [$\delta_{as}(CH_3)$], 1386 m [$\delta_s(CH_3)$], 1007 s, 993 s [(P)O–C], 913 m (O–C, OC–C), 671 s (P=S), 587 m (P–S). ^1H NMR (acetone-d_6): δ = 0.85 s (6H, C^9H_3), 0.95 s (6H, $C^{10}H_3$), 1.06 s (6H, C^8H_3), 1.39 m (4H, C^5H_2), 1.52 m (4H, C^6H_2), 1.63 m (4H, C^3H_2) 1.89 m (2H, C^4H), 3.56 dt (2H, POC^2H, $^3J_{HH}$ = 6.1 Hz, $^3J_{PH}$ = 10.9 Hz), 7.49 m (1H, $C^{5'}H$), 8.31 d (1H, $C^{4'}H$, $^3J_{HH}$ = 6.9 Hz), 8.76 m (1H, $C^{6'}H$), 9.16 s (1H, $C^{2'}H$) ppm. ^{13}C NMR (acetone-d_6, in parentheses is a view of signal in ^{13}C-$\{^1H\}$ NMR): δ = 20.0 q (s) (C^8H_3, $^1J_{CH}$ = 125.4 Hz), 21.0 q (s) (C^9H_3, $^1J_{CH}$ = 125.4 Hz), 22.3 q (s) ($C^{10}H_3$, $^1J_{CH}$ = 125.5 Hz), 26.9 t (s) (C^5H_2, $^1J_{CH}$ = 124.0 Hz), 27.4 t (s) (C^6H_2, $^1J_{CH}$ = 124.0 Hz), 31.1 t (s) (C^3H_2, $^1J_{CH}$ = 124.0 Hz), 42.0 d (s) (C^4H, $^1J_{CH}$ = 135.0 Hz), 49.0 s (s) (C^7), 49.1 s (s) (C^1), 87.0 (d) (HC^2OP, $^2J_{CP}$ = 7.7 Hz), 124.3 (s) ($C^{5'}H$), 126.0 (s) ($C^{3'}$), 137.7 (s) ($C^{4'}H$), 151.6 (s) ($C^{6'}H$), 154.0 (s) ($C^{2'}H$), 165.8 (s) ($C^{7'}$=O) ppm. $^{31}P\{^1H\}$ NMR (ethanol): δ = 114.8 ppm. Elemental analysis for $C_{26}H_{39}NO_4PS_2K$ calculated (%): C, 55.39; H, 6.97; N, 2.48; P, 5.49; S, 11.38; found (%): C, 55.73; H, 7.23; N, 2.12; P, 5.12; S, 11.64.

Biological assay: *in vitro* antimicrobial activities of 1 % concentrations in DMSO of **3b**, **7a**, **7b**, **8a** and **7c** were studied using gel diffusion test on Mueller-Hinton agar and the museum strains of bacterial and fungal cultures of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* (ATCC 25922), *Bacillus cereus* and *Candida albicans* by the Department of Microbiology of Kazan State Medical Academy. Daily cultures of were washed with physiological solution from beef nutrient agar and standardized according to the turbidity standard up to 0.5 by McFarland (1.5 × 10^8 CFU mL^{-1}). Daily cultures of microorganisms were washed with physiological solution from beef nutrient agar. These cultures standardized according to the turbidity standard up to 0.5 by McFarland (1.5 × 10^8 CFU mL^{-1}). Cultures of microorganisms (0.4 mL) were added to melted 10 mL of Mueller-Hinton agar at 45 °C. The mixture obtained was stirred and then poured on sterile Petri dishes (90 mm). The mixture was solidified. Agar plate was punched with a sterile borer (diameter is 6 mm). The obtained holes in agar plate were filled with the test substances. A disinfectant Slayt was used in 1 % concentration. Petri dishes were incubated in incubator for 24–48 h at 35 °C. The diameter of the growth inhibition zones on sterile Petri dishes was measured with an accuracy of ± 0.1 mm.

Detection methods: The $^{31}P\{^1H\}$ NMR spectra were recorded with a Bruker Avance-400 (161.9 MHz) instruments (Bruker BioSpin AG, Fällanden, Switzerland) in benzene or ethanol with 85 % H_3PO_4 as an external reference. The ^1H (400 MHz), $^{13}C\{^1H\}$ (100.6 MHz) spectra were run on a Bruker Avance (III) 400 instruments (Bruker BioSpin AG, Fallanden, Switzerland) in acetone-d_6, CD_3OD or $CDCl_3$ at ambient temperature. Chemical shifts (δ) are given in parts per million (ppm) relative to residual resonance of solvent. Coupling constants (*J*) are given in Hertz (Hz). Fourier transform IR spectra (FTIR) (KBr tablets or films) were obtained with a Bruker Tensor 27 infrared spectrophotometer (Bruker BioSpin AG, Fällanden, Switzerland) (400-4000 cm^{-1}). The ESI mode high-resolution mass spectra (HRMS) were recorded with a Bruker Compass DataAnalysis 4.0 mass spectrometer (Bruker Daltonik Gmbh, Bremen, Germany) in acetone. Melting points were measured by an Electrothermal IA9000 series digital melting point apparatus (Bibby Scientific Ltd., Staffordshire, Great Britain) and uncorrected. The phosphorus contents were determined by pyrolysis method on a non-serial instrument. The determination of the carbon, hydrogen, nitrogen and sulfur composition was performed on a EuroEA3000 CHNS-O Analyzer (EuroVector S.p.A., Milano, Italy).

RESULTS AND DISCUSSION

Taking into account the facile occurrence of the reactions of pyridoxine, nicotine and nicotinamide with dithiophosphoric acids on the basis of monoterpenols [13,14], we decided to vary *O*-organyl substitutes on the phosphorus atom of dithiophosphate anion in their pyridinium derivatives to expand their antimicrobial properties. The chemistry and biological activity of cyclic dithiophosphoric acids derived from diols have been rather little explored [17]. It should be noted that dithiophosphoric acids containing the alkylene chains are rather strong acids. Their pK_a values are quite similar (2.65-2.67) to that of linear dithiophosphoric acids [17]. So alkylene dithiophosphoric acids can form corresponding salts. Thus, ammonium salts of alkylene dithiophosphoric acids were obtained by passing dry ammonia through their benzene solutions [17]. Meanwhile, the chemical behaviour of cyclic dithiophosphoric acids remained practically not studied toward organic nitrogen heterocycles. The present study is concerned with 2-mercapto-2-thiono-1,3,2-dioxaphosphorinanes containing branched alkylene chains to obtain salts with pyridine alkaloids. In this connection, we used 5,5-dimethyl-2-mercapto-2-thiono-1,3,2-dioxaphosphorinane (**1a**) and 4-methyl-2-mercapto-2-thiono-1,3,2-dioxaphosphorinane (**1b**) prepared according to published procedure [15]. The reaction of phosphorinanes (**1a,b**) with (*S*)-(–)-nicotine (**2**) in benzene for 1.5 h at 50 °C has brought about the formation of (*S*)-(–)-nicotinium-2-mercapto-2-thiono-1,3,2-dioxaphosphorinanes (**3a,b**) (Fig. 1).



R^1 = H, R^2 = R^3 = CH_3 (**1a, 3a**); R^1 = CH_3, R^2 = R^2 = H (**1b**, **3b**)

Fig. 1. Synthesis of salts **3a,b**

The products **3a,b** were semisolids similar to other phosphorylated ionic liquids [18-21]. Thus, resultant products are commonly ionic liquids. The reaction of (*S*)-(–)-nicotine **2** with

1,3,2-dioxaphosphorinane **1a** produced new peak in the $^{31}P\{^{1}H\}$ NMR spectrum in benzene which consists a singlet at δ = 108.7 ppm, which is typical for the salts of dithiophosphoric acids [22]. The $^{31}P\{^{1}H\}$ NMR spectrum **3b** in benzene reveals a resonance at the same region (δ = 108.0 ppm). In the FTIR spectra of **3a,b** the absorption bands at 2690–2687 cm^{-1} are assigned to the NH^+ stretching vibrations similarly [23]. The ^{1}H NMR spectra of **3a,b** in CD_3OD show a singlet at δ = 2.76 ppm due to protons of the $NC^{6'}H_3$ substituent of the pyrrolidino cycle of cation. The ESI mass-spectrum (acetone) of **3b** reveals mass peak *m/e* 368.4 attributed to the ion $[M + Na]^+$ (calculated *M* is 346.4). These spectral data indicate an increase in the coordination number of the nitrogen atom of (*S*)-(–)-nicotine to form nicotinium cation. It should be emphasized that (*S*)-(–)-nicotine contains pyridino and pyrrolidino cycles with two nitrogens possessed different basicity [24-26]. (*S*)-(–)-Nicotine has been reported to react with mineral and organic acids to give stable salts with more basic pyrrolidino nitogen [25]. Consequently, increasing in the coordination number of nitrogen of the pyrrolidino group of (*S*)-(–)-nicotine is expected in the reaction with alkylene dithiophosphoric acids.

It is of interest to vary pyridine alkaloids, on the one hand and *O*-organyl substitutes on the phosphorus atom of dithiophosphoric acids, on the other hand, to obtain new pyridinium salts. So we decided to use nicotinic acids and their isomers and derivatives as pyridine alkaloids and involve them in the reactions with dithiophosphoric acids obtained on the basis of monoterpenyl alcohols [15,16]. The reaction of picolinic acid (**2a**) with *O,O*-di[(–)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohex-1-yl] dithiophosphoric acid (**4a**) based on (*S*)-(–)-menthol [15] proceeded in anhydrous ethanol for 1.5 h at 60 °C and resulted in the formation of 2-carboxypyridinium *O,O*-di[(–)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohex-1-yl] dithiophosphate (**6a**) as white solid (Fig. 2). The formation of salt of similar structure could be expected by the reaction of nicotinic acid (**5b**) with *O,O*-di[(1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl) dithiophosphoric acid (**4b**) prepared from (1*R*)-*endo*-(+)-fenchyl alcohol [16]. In fact, 3-carboxypyridinium *O,O*-di[(1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl) dithiophosphate (**6b**) as white solid resulted from the reaction of **4b** with nicotinic acid (**5b**) in ethanol for 2 h at 50 °C (Fig. 2). It should be noted that acid (**4b**) reacts with potassium pyridine-3-carboxylate at room temperature in ethanol for 1 h to form potassium pyridinium-3-carboxylate *O,O*-di[(1*R*)-*endo*-(+)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl) dithiophosphate (**6c**) as white solid (Fig. 2). As we can see, the reactivity of potassium salt of nicotinic acid is higher than that of nicotinic acid itself toward dithiophosphoric acid (**4b**).

It is considered of interest to compare the spectral parameters of salts **6a**, **6b** and **6c** possessed the similar structure of pyridinium cation. The $^{31}P\{^{1}H\}$ chemical shift value of potassium pyridinium-3-carboxylate dithiophosphate (**6c**) is shifted down field (δ = 114.8 ppm) in comparison with the signals (δ = 101.3–106.4 ppm) of pyridinium salts **6a** and **6b** containing carboxylic groups. However, in the FTIR spectra of salts **6b-c** the positions of weak bands (2726–2706 cm^{-1}) assigned to the NH^+ stretching vibrations are not affected by changing position of carboxylic substituents of pyridinium cation. The best evidence for the formation of salt **6a-c** is provided by the ESI mass-spectra. Thus, ESI mass-spectrum of **6a** shows peak of *m/e* 568.6 due to the ion $[M + K]^+$ (calculated *M* 529.7.). In the ESI mass-spectrum of **6b** the peak of *m/e* 1072.2 is assigned to the ion $[2M + Na]^+$ (calculated *M* 525.7). Thus, picolinic and nicotinic acids and its potassium salt give corresponding 2-carboxypyridinium, 3-carboxypyridinium and potassium pyridinium-3-carboxylate dithiophosphates.

In recent study, nicotinium and nicotinamide salts of dithiophosphoric acids were evaluated as antibacterials against clinically relevant infection by dilution method to evaluate MIC sterile Mueller-Hinton broth [13]. However, results based on the hole method have not been previously obtained. In present study, salt **3b** and pre-synthesized (*S*)-(–)-nicotinium *O,O*-di(2-isopropyl-5-methylphenyl) dithiophosphate (**7a**), (*S*)-(–)-nicotinium *O,O*-di[(1*S*,2S,3*S*,5*R*)-(+)-2,6,6-trimethylbicyclo[3.1.1]-hept-3-yl] dithiophosphate (**7b**), nicotinamide salt of *O,O*-di(2-isopropyl-5-methylphenyl) dithiophosphoric

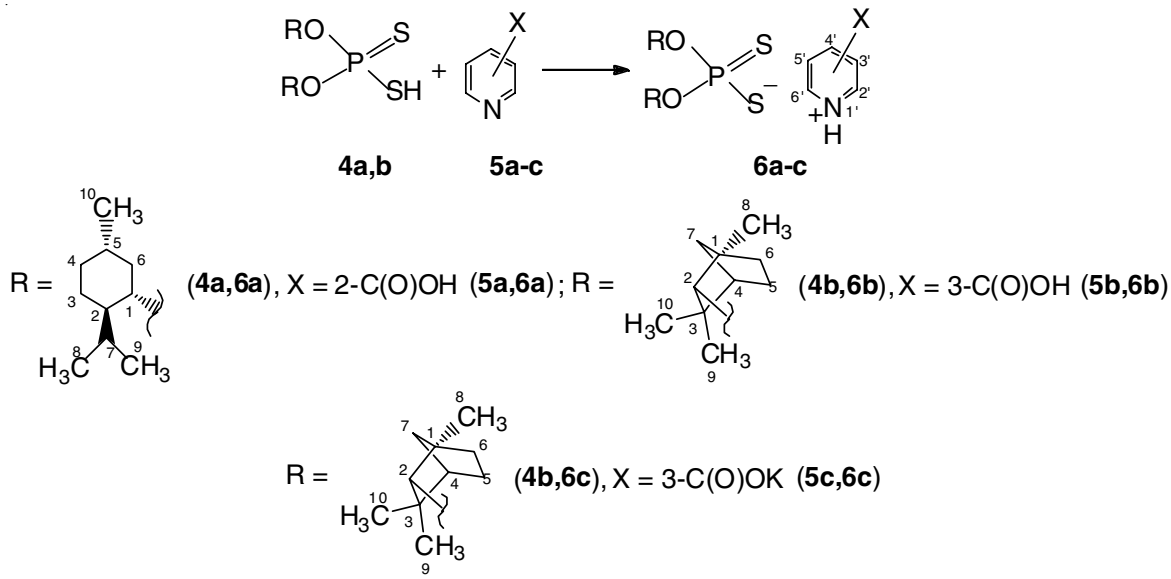


Fig. 2. Synthesis of salts **6a-c**

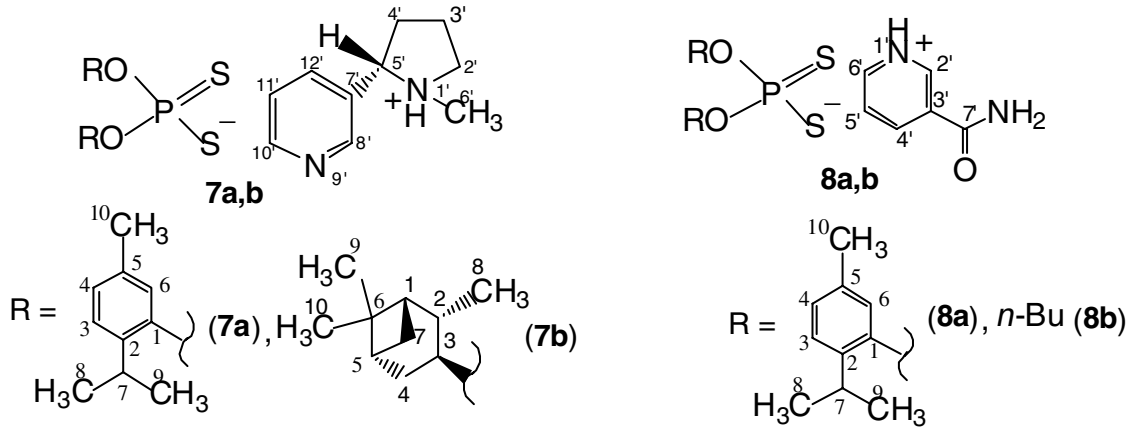


Fig. 3. (*S*)-(–)-Nicotinium and nicotinamide salts of dithiophosphoric acids **7a,b** and **8a,b**

acid (**8a**) and nicotinamide salt of *O,O*-dibutyl dithiophosphoric acid (**8b**) [13] (Fig. 3) (concentration in DMSO was 1 %) were evaluated for their *in vitro* antibacterial and antifungal activity against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* (ATCC 25922), *Bacillus cereus* and *Candida albicans*. The results are given in Table-1. Control sample was disinfectant Slayt (1 %) [27]. Inspection of Table-1 proves that all prepared compounds showed remarkable activity toward the tested strains of *S. aureus* (growth inhibition zones of 12-30 mm). Compounds **7a** and **7b** on the basis of (*S*)-(–)-nicotine show greater activity against all tested microorganisms. *S. aureus* proved to be the most sensitive toward compound **7a** (30 mm). All tested salts exhibit moderate activity against *E. coli* (8-16 mm). Salt **8a** prepared from nicotinamide inhibits the growth of *E. coli* on 20 mm. It was noticed that the tested compounds significantly inhibited the high activity against fungi *C. albicans* (15-16 mm) in compare with disinfectant Slayt (14 mm). Nicotinamide salt of *O,O*-dibutyl dithiophosphoric acid (**8b**) contaning *O*-alkyl substituents and nicotinamide cation showed low activity against all tested microorganisms (10-13 mm).

TABLE-1
ANTIMICROBIAL ACTIVITY OF SALTS **3a**, **7a**, **7b**, **8a** AND **8b**[a]

Compound	*S. aureus*	*E. coli*	*B. cereus*	*C. albicans*
3b	17	8	13	16
7a	30	12	26	15
7b	27	16	28	16
8a	15	20	24	15
8b	12	10	13	10
Slayt [27][b]	19	10	12	14

[a]In 1 % concentrations7 in DMSO; [b]In 1 % concentration.

Thus, the higher activity of the compounds **7a** and **7b** is mainly due to contain *O*-monoterpenyl substituents on the phosphorus atom as well as nicotinium cation which are responsible for the enhanced activity of these compounds.

Conclusion

Cyclic dithiophosphoric acids readily form (*S*)-(–)-nicotinium alkylene dithiophosphates on the basis of substituted 2-mercapto-2-thiono-1,3,2-dioxaphosphorinanes. These reactions proceed *via* increasing in the coordination number of nitrogen of the pyrrolidino cycle of (*S*)-(–)-nicotinium cation. Synthesis of new 2-carboxypyridinium, 3-carboxypyridinium and potassium pyridinium-3-carboxylate *O,O*-diterpenyl dithiophosphates are of interest from the point of view of preparative organophosphorus chemistry for obtaining new ionic liquids. The reactivity of potassium salt of nicotinic acid is higher than that of nicotinic acid itself toward the same dithiophosphoric acid. This study revealed that the *O*-monoterpenyl substituents on the phosphorus atom of dithiophosphate anion and pyridinium cation could be useful as template for future, development through modification of dithiophosphoric acids to design a more potent antimicrobial agents.

ACKNOWLEDGEMENTS

The authors are grateful to the staff of Distributed Spectral-Analytical Center of Shared Facilities for Study of Structure, Composition and Properties of Substances and Materials of Federal Research Center of Kazan Scientific Center of Russian Academy of Sciences for their research and assistance in discussing the results.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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