Synthesis, Anticancer and Antimicrobiological Activities of Pyrrolo[2,3-*d*]pyrimidines

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Reactions of 6-amino-3,4-dihydro-2-methoxy-4-oxopyrimidine **1a** and its 3-methyl derivative **1b** with chloroacetaldehyde and chloroacetyl chloride are discussed in this paper. Amongst others compounds, we have obtained, in low yield, the novel ring system oxazolo[3,2-*c*]pyrrolo[3,2-*e*]pyrimidine. The anticancer and antimicrobial activities of some of the obtained products are described.

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Pyrrolo[2,3-*d*]pyrimidines (7-deazapurines) and furo-[2,3-*d*]pyrimidines are an important class of compounds, structurally and chemically related to naturally nucleosides and some antibiotics [1]. The biological activity of these compounds is well known and they have been intensively investigated as antitumor, antiallergic, antiviral, antiinflammatory etc. agents [2]. Due to the interest of this class of compounds we have previously reported reactivity and synthetic applications of 6-aminopyrimidines [3] and 6-glycosylaminopyrimidines [4]; as a continuation of them, in the present paper we describe the reactions of two 6-amino-4-oxopyrimidines **1a,b** with chloroacetaldehyde and chloroacetyl chloride as well as the results of anticancer "in vivo" tests against L-1210 Leukemia of the obtained compounds and their antimicrobial activity against several strains of bacteria and yeasts.

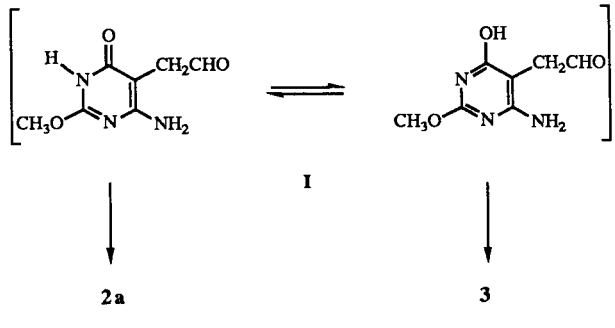
The reaction of **1a** with an excess of chloroacetaldehyde in water and sodium acetate led after five minutes under reflux to **2a** (60%), **3** (6%) and **4** (7%) (Scheme I). The compound **2a** has been previously prepared by Seela *et al.* [5] in 46% yield by condensation between *O*-methyluronium sulfate and ethyl 2-cyano-4,4-diethoxybutanoate in sodium methoxide/methanol followed by acid treatment.

The reaction of **1b** with chloroacetaldehyde by using the same conditions described for **1a**, led, as unique product, to **2b** in 60% yield. In the ^{1}H-nmr spectrum of **2b**, the signals assigned to H-5 and H-6 appears as double doublet due to the coupling with N(7)-H. These signals becomes to doublets by adding deuterium oxide. In the spectrum of **2a**, a similar coupling system is observed.

We have not found bibliographic accounts about the tricyclic compound **4**. The analytical and spectroscopic data are consistent with the proposed structure for this compound. Thus, the ^{1}H-nmr espectrum of this compound shows four doublets assigned to H-2, H-8, H-9 and H-3 and one singlet assigned to the CH_3-O group. In the ^{13}C-nmr spectrum, the signals due to C-2, C-3, C-8 and C-9 atoms have been assigned using DEPT technique.

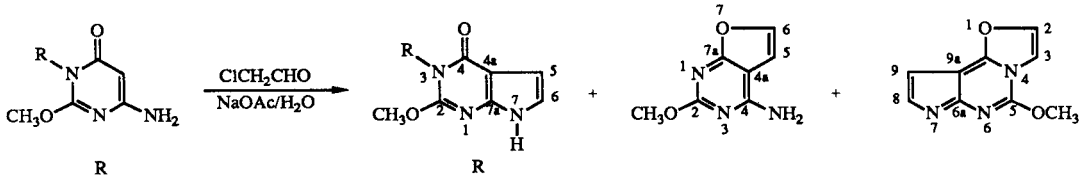
The reaction of **1a** and **1b** with chloroacetaldehyde can pass through the intermediate **I** (Scheme II) formed by attack at C-5 position. The intermediate **I** can follow two different pathways to give the final products: formation of **3**

Scheme II

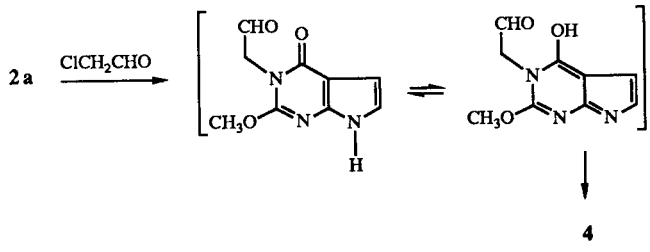


by intramolecular cyclization "*via*" C(4)-OH and formation of **2a** or **2b** by intramolecular cyclization "*via*" C(6)-NH_2. The higher yield of compound **2a** than **3** would be due to the strong interaction between the carbonyl group, in compound **1a**, and the solvent (water) through

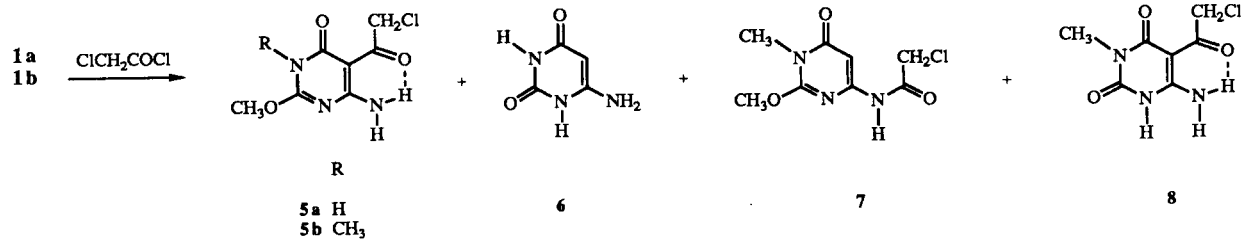
Scheme I



Scheme III



Scheme IV



intermolecular hydrogen bond which inactivates the mentioned group for an intramolecular nucleophilic addition [6].

Compound **4** can be formed by *N*-alkylation of **2a** (chloroacetaldehyde is in excess) and subsequent intramolecular cyclization towards C(4)-OH as shows in Scheme III. In the reaction of 6-aminouracil and its alkylated derivatives with chloroacetaldehyde, 7-deazapurines have been obtained as unique products [7].

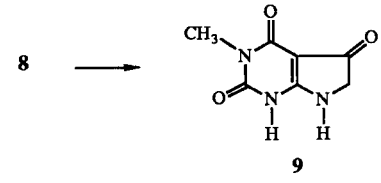
The reaction of **1a** and **1b** with an excess of freshly distilled chloroacetyl chloride were carried out in both anhydrous ethyl acetate and chloroform as solvent under reflux. Compound **1a** afforded **5a** (2%) and the known 6-aminouracil **6** (12%) [8]. Compound **1b** led 73% yield of **5b**, 2% yield of **7** and 18% yield of **8** (Scheme IV). The reactions were not completed and starting product, **1a** and **1b**, were recuperated unreacted; however, significantly better yields were obtained when chloroform was used as solvent as we describe in the experimental part. Higher yield are obtained for the reaction of **1b** due to its larger solubility in chloroform.

In the ^{1}H-nmr spectra of **5a**, **5b** and **8**, the downfield shifting of one of the hydrogen atoms of the C(6)-NH_2 group can be attributed to the six membered hydrogen bond formation as is shown in the Scheme IV. In the mass spectra of **5b** and **7**, the usual isotopic distribution for the molecular peak due to the presence of one chlorine atom is observed.

The reaction of **1a** and **1b** with chloroacetyl chloride take place by the acylation on C-5 position of the pyrimidine ring to afford **5a** and **5b**. In this reaction a molecule of hydrogen chloride is produced and induces formation of **6** and **8** by hydrolysis of the CH_3-O group as we have observed in similar cases [9]. The lone electronic pair of nitrogen of the C(6)-NH_2 group is less required for the ring moiety due to the presence of N(3)-CH_3 in compound **1b**; this makes possible the acylation which yields **7**.

Cyclization of **8** to **9** has been carried out with potassium carbonate in DMF at 80° (Scheme V). Compound **5b** afforded a complex mixture which was impossible to resolve under the conditions above mentioned.

Scheme V



Compounds **2a**, **2b**, **5b** and **8** have been tested "*in vivo*" as inhibitors of the L-1210 Leukemia at the National Cancer Institute (NCI) according to standard methods. The T/C percent values have ranged between 101 (**2b**, 60 mg/Kg) and 86 (**2a**, 120 mg/Kg) and none of these products have shown significant anticancer activity. Compound **8** was toxic at 240 and 120 mg/Kg.

The antimicrobial activity of **2a**, **2b**, **4**, **5b**, **6**, **8** and **9** against some bacteria and yeast strains has been investigated. Compound **4** has shown some activity towards genus *Staphylococcus* (MIC 50 μg/ml), *Bacillus* (MIC 100 μg/ml) and *Candida* (MIC 100 μg/ml), however this compound has shown a lack of inhibitory activity in other microorganisms. The other compounds have presented a weak or lack antimicrobial activity against Gram positive bacteria and yeast especially.

EXPERIMENTAL

Melting points were determined using a Melting Point Apparatus Gallenkamp and are uncorrected. Proton nuclear magnetic resonance spectra were recorded with a Hitachi Perkin-Elmer R-600 and a Bruker AM-300 spectrometers using tetramethylsilane as internal standard. Chemical shifts were expressed in δ values. The following abbreviations were used; s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; dd, double doublet. Carbon-13 nuclear magnetic resonance spectra were determined with a Bruker AM-300 spectrometer. Ultraviolet spectra were recorded with a Perkin-Elmer Lambda 5 spectrophotometer. The following abbreviation was used: sh, shoulder. Infrared spectra were recorded using a Beckman 4250 spectrophotometer (potassium bromide pellets). The following abbreviations were used: m, medium; st, strong; w, weak. The analysis of C, H and N have been performed in the "Servicios Técnicos de la Universidad de Granada" using a Perkin-Elmer 240C equipment. Mass spectra were recorded using a Hewlett-Packard HP-5988-A spectrometer. Thin layer chromatography (tlc) was ran on silica gel Merck 60 GF_{254}, visualization was accomplished by ultraviolet absorbance. Finally, column chromatography was done on Kieselgel 60 silica gel (70-230 mesh) using the solvent systems indicated in each case.

Reaction of **1a** with Chloroacetaldehyde.

To a solution of 1.41 g (10 mmoles) of **1a** and 0.82 g (10 mmoles) of sodium acetate in 40 ml of boiling water, an aqueous solution of 2.34 ml (20 mmoles) of chloroacetaldehyde was added. The mixture was stirred under reflux for 5 minutes; at this time starting product was not detected in tlc (dichloromethane/ethanol 6:1). The white solid which precipitated was filtered, washed with cold water and recrystallized from ethanol. This product was identified as 3,4-dihydro-2-methoxy-4-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine **2a**, mp 254-258°, (lit [5] mp 260°).

The mother liquors of the above reaction were extracted with dichloromethane (5 × 10 ml), the organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. To the obtained residue, 1 g of silica gel and 20 ml of hexane were added; the mixture was then evaporated under reduced pressure, poured into a chromatographic column which contain 100 g of silica gel and eluted with dichloromethane/ethanol (0-1.5% in ethanol) mixtures. The first collected fraction was recrystallized from ethyl ether and identified as 5-methoxyoxazolo-[3,2-*c*]pyrrolo[3,2-*e*]pyrimidine **4**, 0.14 g (7%), mp 110-112° dec; ^{1}H-nmr (DMSO-d_6): 7.90 (1H, d, J = 3 Hz, H-2), 7.80 (1H, d, J = 2 Hz, H-8), 7.50 (1H, d, J = 2 Hz, H-9), 7.15 (1H, d, J = 3 Hz, H-3), 4.20 (3H, s, CH_3-O); ^{13}C-nmr (DMSO-d_6): 155.83, 147.02, 142.42 (C-9, C-5, C-6a), 141.63 (C-2), 132.56 (C-3), 109.51 (C-8), 104.76 (C-9), 99.36 (C-9a), 56.25 (CH_3-O); ir: 3150 (m), 3125 (m), 1635 (st), 1545 (st, br), 1510 (st), 1375 (st), 1320 (st), 1150 (st) 1080 (m), 945 (m), 720 (m); uv (methanol): λ max (nm) (ε) 224 (21200), 272 (9700), 289 (sh); ms: 190 (M^++1), 189 (M^+).

Anal. Calcd. for $C_9H_7N_3O_2$: C, 57.14; H, 3.73; N, 22.21. Found: C, 57.11; H, 4.03; N, 21.96.

The second collected fraction was recrystallized from ethyl ether and identified as 4-amino-2-methoxyfuro[2,3-*d*]pyrimidine **3**, 0.10 g (6%), mp 155-156° dec; ^{1}H-nmr (DMSO-d_6): 7.50 (1H, d, J = 2 Hz, H-6), 7.40 (2H, s, br, exchangeable with deuterium oxide, NH_2), 6.90 (1H, d, J = 2 Hz, H-5), 3.80 (3H, s, CH_3-O); ^{13}C-nmr (DMSO-d_6): 168.14, 163.18, 159.46 (C-7a, C-2, C-4), 139.49 (C-6), 104.35 (C-5), 95.02 (C-4a), 53.92 (CH_3O); ir: 3390 (st), 3310 (m), 3120 (st, br), 1645 (st), 1590 (st), 1530 (m), 1460 (m), 1360 (st), 1140 (m), 1090 (m), 1015 (m), 745 (m); uv (methanol): λ max (nm) (ε) 220 (5540), 255 (9600), 269 (sh).

Anal. Calcd. for $C_7H_7N_3O_2$: C, 50.91; H, 4.27; N, 25.44. Found: C, 50.90; H, 4.20; N, 25.38.

The third isolated product was an additional amount of **2a**, total yield 0.99 g (60%).

Reaction of **1b** with Chloroacetaldehyde.

To a solution of 1.55 g (10 mmoles) of **1b** and 0.82 g (10 mmoles) of sodium acetate in 40 ml of boiling water, an aqueous solution of 2.34 ml (20 mmoles) of chloroacetaldehyde was added. The mixture was stirred under reflux for 5 minutes; at this time starting product was not detected in tlc (benzene/ethanol, 3:2). After 10-12 hours at room temperature a white solid precipitated, this was filtered, washed with cold water and recrystallized from ethanol being identified as 3,4-dihydro-3-methyl-2-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine **2b**: 1.25 g (70%), mp 265-267° dec; ^{1}H-nmr (DMSO-d_6): 11.40 (1H, s, br, exchangeable with deuterium oxide, N(7)-H), 6.85 (1H, dd, + D_2O → d, J = 3.7 Hz, H-6), 6.40 (1H, dd, + D_2O → d, J = 3.7 Hz, H-5), 4.05 (3H, s, CH_3O), 3.40 (3H, s, CH_3N); ^{13}C-nmr (DMSO-d_6): 158.16 (C-2), 153.84 (C-4), 146.08 (C-7a), 118.65 (C-6), 101.95 (C-5), 101.55 (C-4a), 55.43 (CH_3O), 27.19 (CH_3N); ir: 3190 (m), 1650 (st), 1555 (st, br), 1510 (m), 1410 (m), 1335 (m), 1200 (m), 985 (m), 880 (m), 785 (m), 620 (m); uv (methanol): λ max (nm) (ε) 221 (6900), 254 (8000), 272 (sh).

Anal. Calcd. for $C_8H_9N_3O_2$: C, 53.62; H, 5.06; N, 23.45. Found: C, 53.83; H, 5.03; N, 23.39.

Reaction of **1a** and **1b** with Chloroacetyl Chloride.

To a suspension of 10 mmoles of **1a** or **1b** in 100 ml of anhydrous chloroform, 1.62 ml (20 mmoles) of freshly distilled chloroacetyl chloride were added. The mixture was stirred under reflux for 10 hours. After this time the reaction mixture was evaporated under reduced pressure. To the residue obtained, 2 g of silica gel and 20 ml of hexane were added; the mixture was then evaporated, poured into a chromatographic column which contained 100 g of silica gel and eluted with dichloromethane/ethanol (0-25% in ethanol for **1a** and 0-5% in ethanol for **1b**) mixtures.

In the reaction of **1a** two products were obtained. The first collected fraction was recrystallized from ethyl ether and identified as 6-amino-5-(α-chloroacetyl)-3,4-dihydro-2-methoxy-4-oxopyrimidine **5a**, 0.04 g (2%), mp >300° dec; ^{1}H-nmr (DMSO-d_6): 11.90 (1H, s, br, exchangeable with deuterium oxide, N(3)-H), 9.70 (1H, s, br, exchangeable with deuterium oxide, C(6)-NH), 8.40 (1H, s, br, exchangeable with deuterium oxide, C(6)-NH), 4.95 (2H, s, $COCH_2$), 3.95 (3H, s, CH_3O); ^{13}C-nmr (DMSO-d_6): 189.05 (C=O), 165.92, 163.10, 158.23 (C-4, C-2, C-6), 92.33 (C-5), 54.97 (CH_2), 51.12 (CH_3-O); ir: 3495 (m), 3290 (m), 2800 (w, br), 1655 (st), 1610 (st, br), 1530 (st), 1485 (m), 1340 (m), 1310 (m), 1215 (m), 1190 (m), 1100 (w), 1015 (w), 785 (m), 640 (m); uv (methanol): λ max (nm) (ε) 224 (22900), 257 (sh), 281 (11100).

Anal. Calcd. for $C_7H_8ClN_3O_3$: C, 38.63; H, 3.70; N, 19.31. Found: C, 38.59; H, 3.96; N, 19.28.

The second collectd fraction was recrystallized from DMSO-H_2O being identified as 6-amino-2,4-dioxo-1,2,3,4-tetrahydropyrimidine **6**, 0.15 g (12%), mp >300° dec (lit [8] mp >300° dec).

After column chromatography, the reaction of **1b** led to three products. The first one was recrystallized from ethanol and identified as 6-amino-5-(α-chloroacetyl)-3,4-dihydro-3-methyl-2-meth-

oxy-4-oxopyrimidine **5b**, 1.70 g (73%), mp 183°; ^{1}H-nmr (DMSO-d_6): 9.60 (1H, s, br, exchangeable with deuterium oxide, C(6)-NH), 8.30 (1H, s, br, exchangeable with deuterium oxide, C(6)-NH), 4.90 (2H, s, $COCH_2$), 4.10 (3H, s, CH_3-O), 3.30 (3H, s, CH_3-N); ^{13}C-nmr (DMSO-d_6): 163.75, 162.02, 157.40 (C-4, C-2, C-6), 92.26 (C-5), 56.00 (CH_3O), 51.44 (CH_2), 26.94 (CH_3-N); ir: 3380 (m), 3260 (w), 1635 (st), 1610 (st), 1580 (st), 1510 (st), 1385 (w), 1240 (m), 1215 (m), 945 (w), 775 (m), 640 (m); uv (methanol): λ max (nm) (ε) 226 (23200), 288 (11300); ms: 233 (M^++2), 232 (M^++1), 231 (M^+).

Anal. Calcd. for $C_8H_{10}ClN_3O_3$: C, 41.48; H, 4.35; N, 18.14. Found: C, 41.73; H, 4.29; N, 18.56.

The second fraction was recrystallized from ethanol and identified as 6-chloroacetamido-3,4-dihydro-3-methyl-2-methoxy-4-oxopyrimidine **7**, 0.05 g (2%), mp 212-214°; ^{1}H-nmr (DMSO-d_6): 10.05 (1H, s, exchangeable with deuterium oxide, C(4)-NH), 6.65 (1H, s, H-5), 4.35 (2H, s, CH_2), 3.95 (3H, s, CH_3-O), 3.20 (3H, s, CH_3-N); ^{13}C-nmr (DMSO-d_6): 166.06 (*C*OCH2), 162.84, 156.56, 153.52 (C-6, C-2, C-4), 90.95 (C-5), 55.82 (CH_3-O), 43.32 (CH_2), 27.12 (CH_3-N); ir: 3360 (w), 1650 (st, br), 1535 (st, br), 1410 (m), 1240 (m), 1205 (m), 1010 (w), 970 (w); uv (methanol): λ max (nm) (ε) 226 (21400), 281 (7820); ms: 233 (M^++2), 232 (M^++1), 231 (M^+).

Anal. Calcd. for $C_8H_{10}ClN_3O_3$: C, 41.48; H, 4.35; N, 18.14. Found: C, 41.39; H, 4.53; N, 17.93.

The third isolated product was recrystallized from ethanol being identified as 6-amino-5-(α-chloroacetyl)-2,4-dioxo-3-methyl-1,2,3,4-tetrahydropyrimidine **8**, 0.39 g (18%), mp >300° dec; ^{1}H-nmr (DMSO-d_6): 11.10 (1H, s, br, exchangeable with deuterium oxide, N(1)-H), 9.70 (1H, s, br, exchangeable with deuterium oxide, C(6)-NH), 7.30 (1H, s, br, exchangeable with deuterium oxide, C(6)-NH), 4.80 (2H, s, CH_2), 3.05 (3H, s, CH_3-N); ^{13}C-nmr (DMSO-d_6): 162.27; 156.98, 148.91 (C-6, C-2, C-4), 88.92 (C-5), 51.04 (CH_2), 26.33 (CH_3-N); ir: 3420 (m), 3240 (m), 3200 (m), 1720 (m), 1650 (st, br), 1510 (m), 1450 (m, br), 1220 (m), 750 (m); uv (methanol): λ max (nm) (ε) 224 (14300), 270 (13400).

Anal. Calcd. for $C_7H_8ClN_3O_3$: C, 38.63; H, 3.71; N, 19.31. Found: C, 38.80; H, 3.52; N, 19.76.

Cyclization of the 5-(α-Chloroacetyl) Derivative **8**.

To 3 ml of DMF, 0.435 g (2 mmoles) of **8** and 0.28 g (2 mmoles) of anhydrous potassium carbonate were added. The reaction mixture was stirred at 90° for 30 minutes; at this time starting product was not detected in tlc (dichloromethane/ethanol, 6:1). To the reaction mixture, 3 g of silica gel and 20 ml of hexane were added, the mixture was then evaporated under reduced pressure, poured into a chromatographic column which contain 50 g of silica gel and eluted with hexane. When the DMF was eliminated, the compound was eluted using dichloromethane/ethanol (0-2% in ethanol) mixtures. The eluted fractions containing the compound were evaporated under reduced pressure and recrystallized from ethanol being identified as 3-methyl-2,4,5-trioxo-1,2,3,4,5,6-hexahydro-7*H*-pyrrolo[2,3-*d*]pyrimidine **9**, 0.25 g (70%), mp >300° dec; ^{1}H-nmr (DMSO-d_6): 8.00 (1H, s, br, exchangeable with deuterium oxide, N(1)-H), 6.95 (1H, s, br, exchangeable with deuterium oxide, N(7)-H), 4.90 (2H, s, CH_2), 3.20 (3H, s, CH_3-N); ^{13}C-nmr (DMSO-d_6); 188.43 (C-5), 177.27 (C-4), 166.50 (C-2, C-4a), 158.89 (C-7a), 77.59 (C-6), 27.45 (CH_3-N); ir: 3490 (m), 3420 (m), 3350 (m), 3170 (m), 1690 (st), 1660 (st), 1640 (st), 1570 (st), 1245 (m), 1180 (m), 780 (m); uv (methanol): λ max (nm) (ε) 227 (22000), 266 (19000).

Anal. Calcd. for $C_7H_7N_3O_3$: C, 46.41; H, 3.89; N, 23.20. Found: C, 46.40; H, 3.81; N, 23.14.

Anticancer and Antimicrobial Activity.

"*In vivo*" antitumor activity against L-1210 Lukemia was determined by the NCI according to the protocol described in instruction 14. The L-1210 Leukemia was implanted into CDF_1 mice and each mouse was inoculated one at various dose levels and observed for 20 d. The result evaluated as %T/C = (median survival time (MST) treated/MST control)×100, and compound is considered active if %T/C exceeds 125.

The determination of minimal inhibitory concentration (MIC) of these compounds against: *Pseudomonas*, *E. coli*, *Proteus*, *Salmonella*, *Micrococcus*, *Staphylococcus*, *Bacillus*, and *Candida* was performed with some modifications of the technique described by R. N. Jones *et al.* [10].

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