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SYNTHESIS OF SOME HETROCYCLES FROM DEHYDRO-L-ASCORBIC ACID AND THEIR BIOLOGICAL ACTIVITY

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Sythesis of some hetrocyclic compouds from dehydro-L-ascorbic acid, monoaryl hydrazone and their biological activities are described.

Key words: Nitrogenhetrocycles, Dehydro-L-ascorbic acid, Biological activity.

Introduction

In continuation of our work on the synthesis of nitrogen heterocycles from dehydro-L-ascorbic acid and its 5-epimer, *mono-* and *bis*-hydrazones [1-6], we described now the synthesis of some heterocyclic compounds and their biological activity. Dehydro-L-ascorbic acid as well as its monoarylhydrazones are considered excellent precursors for many heterocyclic compounds, e.g., triazoles, imidazoles, isoxazoles and pyrazoles. Some of the 4, 5-pyrazolinediones have shown significant biological activity [7-10], and this attracted our attention towards the synthesis of some heterocycles which might be of some biological interest.

Experimental

General methods. Melting points were determined with a Tottoli (Buchi) apparatus and are uncorrected. Infrared spectra were determined with a 580B Perkin-Elmer spectrometer. ^{1}H-nmr spectra were determined with a Varian EM-390 spectrometer with tetramethylsilane as internal standard. Chemical shifts are given on the scale. Mass spectra were recorded with a LKB 209 spectrometer; intensities are given in parentheses as percentages of the base peak. Microanalyses were carried out in the Department of Chemistry, University of King Fahd, University of Petroleum and Minerals, Dhaharan, Saudi Arabia, and in the Chemistry Department , Kuwait University, Kuwait and Cairo University, Cairo, Egypt.

L-threo-2,3-hexodiulosono-1, 4-lactone 2-(p-nitrophenylhydrazone) (1). A solution of dehydro-L-ascorbic acid obtained by oxidising a solution of L-ascorbic acid (10 g) in water (50 ml) with iodine (13 g) in ethanol (50 ml) was treated with *p*-nitrophenylhydrazine (1 g) in ethanol (50 ml) and few drops of acetic acid. On keeping for 3 days at room temperature, L-threo-2, 3-hexodiulosono-1, 4-lactone 2-(*p*-nitrophenylhydrazone) crystallized out and was filtered, washed with water and ethanol (1:1) and dried (yield 0.8 g). On recrystallization from ethanol it formed yellow needles, m.p. 187-189° (Lit. [4], found: m.p. 189-190°).

L-threo-2,3-Hexodiulosono-1,4-lactone2-(p-nitrophenylhydrazone)-3-semicarbazone (2). A solution of L-threo-2, 3-hexodiulosono-1,4-lactone-2-(*p*-nitrophenylhydrazone) (1) (1 g) in ethanol (30 ml) was treated with semicarbazide hydrochloride (1 g), and sodium acetate (1 g), in acetic acid (5 ml), was boiled under reflux for 5 hr. It was concentrated to a small volume and left to cool at room temperature. The solid that separated was filtered, washed with water and ethanol and dried (yield 0.8 g). On recrystallization from ethanol it formed orange needles, m.p. 250-251°; υ_{max} KBr 3350 (OH), 3120 (NH), 1730 (lactone C=O), 1680 (OCN), and 1600 cm^{-1} (C=N).

Analysis: Calc. for $C_{13}H_{14}N_6O_7$; C, 42.62; H, 3.83; N, 22.95. Found: C, 42.39; H, 3.86; N, 22.76.

3-(L-threo-glycerol-1-yl)-4, 5 (1H)-pyrazolinedione 4-(p-nitrophenylhydrazone) (4). A solution of compound (2) (0.5 g) in concentrated ammonia (30 ml) was heated under reflux for 4 hrs, concentrated to a small volume and left to cool at room temperature. The solid which separated out was filtered, washed with water and ethanol (1:1) and dried (yield 0.4 g). On recrystallization from ethanol it formed orange needles, m.p. 199-200°; υ_{max} KBr 3450 (OH), 3100 (NH), and 1680 cm^{-1} (OCN); *m/z* 323(72), 307(52), 305(36), 292(48) 276(22), 275(37), 262(39), 246(70, 150(100), 137(82), 136(34), 122(26).

Analysis: Calc. for $C_{12}H_{13}N_5O_6$: C, 44.58; H, 4.02; N, 21.67. Found: C, 44.32; H, 4.37; N, 21.92.

1-Acetyl-3-(tri-o-acetyl-L-threo-glycerol-1-yl)-4,5-pyrazolinedione-4-(p-nitrophenylhydrazone).(5). A suspension of compound (4) (0.1 g), in acetic anhydride (10 ml) was boiled under reflux for 1 hr. The mixture was poured onto crushed ice, and the solid filtered, successively washed with water: ethanol (1:1) and ether and dried (yield 60 mg). On recrystalization from ethanol it formed yellow needles, m.p. 170-172°; υ_{max} KBr1740 (ester), 1685 cm^{-1} (OCN); ^{1}H-nmr data ($CDCl_3$);

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δ 2.06, 2.08, 2.20 (3s, 9H, 3 $OCOCH_3$), 2.58 (s, 3H, $NCOCH_3$), 4.35 (m, 2 H, H-3'), 5.78 (m, 1 H, H-2'), 6.18 (d, 1 H, H-1'), 7.62-8.44 (m, 4 H, phenyl), 13.42 (s, 1 H, NH).

Analysis: Calc. for $C_{20}H_{21}N_5O_{10}$. $1/2H_2O$: C, 48.0; H, 4.40; N, 14.0. Found: C, 48.42; H, 3.82; N, 13.92.

Reaction of L-threo-2, 3-hexodiulosono-1, 4-lactone 2-(p-nitrophenylhydrazone) with hydrazine hydrate. To a solution of L-threo-2, 3-hexodiulosono-1, 4-lactone-2-(*p*-nitrophenylhydrazone) (0.1 g) in methanol (25 ml) hydrazine hydrate (2 ml) and a few drops of acetic acid was added. The mixture was boiled under reflux for 2 hrs, and left to cool at room temperature. The solid that separated out was filtered, successively washed with water and ethanol and dried (yield 60 mg). On recrystallization from ethanol it formed orange needles, m.p. 199-200°, alone or mixed with the product obtained from boiling of L-threo-2, 3-hexodiulosono-1, 4,-lactone 2-(*p*-nitrophenylhdrazone) 3-(semicarbazone) with concentrated ammonia. Both products has identical elemental analysis and spectral data.

3-Formy 1-4, 5 (1H)-pyrazolinedione 4-(p-nitrophenylhy-drazone) (8). A suspension of compound (4) (0.1 g) in water (20 ml) was treated with a solution of sodium metaperiodate (0.5 g) in water (10 ml), and the mixture was shaken for 6 hrs, at room temperature. The solid that separated out was filtered, washed with water and ethanol and dried (yield 50 mg). On recrystallization from ethanol it formed orange needles, m.p. 242-243°; υ_{max} KBr 1700 cm^{-1} (OCN and CHO).

Analysis: Calc. for $C_{10}H_7N_5O_4$: C, 45.98; H, 2.68; N, 26.81 . Found: C, 45,47; H, 2.42; N, 26.73.

3-Hydroxymethyl-4,5-(1H)-pyrazolinedione 4-(p-nitrophenylhydrazone (9). To a solution of compound (8) (0.1 g) in methanol (20 ml) was treated with a solution of sodium borohydride (0.1 g) in water (10 ml) was added in small portions with occasional shaking. The solution was acidified with acetic acid, and the solid that separated out was filtered, washed with water and ethanol and dried (yield 60 mg). On recrystallization from ethanol it formed yellow needles, m. p. 195-198°; υ_{max} KBr 3100 (NH), and 1680 -1 (OCN).

Analysis: Calc. for $C_{10}H_9N_5O_4$: C, 45.62; H, 3.42; N, 26.62. Found: C, 45.91; H, 3.52; N, 26.31.

3-Acetoxymethyl-1-acetyl-4,5-pyrazolinedione-4-(p-nitrophenyl hydrazone) (10). A suspension of compound (9) (0.1 g), in acetic anhydride (10 ml) was boiled under reflux for 1 hr. The mixture was poured onto crushed ice, and the product was filtered, sucessively washed with water and ethanol and dried (yield 50 mg). On recrystallization from ethanol it formed yellow needles, m.p. 141-143°; υ_{max} KBr 1750 (ester) and 1680 cm^{-1} (OCN); ^{1}H-nmr data $(CD_3)_2CO$) δ 2.05, (s, 3 H, $OCOCH_3$), 2.58 (s, 3H, $NCOCH_3$), 5.0 (s, 2 H, CH_2), 7.28-8.12 (m, 4 H, aromatic protons), 13.60 (s, 1 H, NH).

Analysis: Calc. for $C_{14}H_{13}N_5O_6$: C, 48.41; H, 3.74; N, 20.17. Found: C, 48.33; H, 3.21; N, 20.43.

Condensation products (11-15) of 3-formyl-4, 5(1H)-pyrazolinedione 4-(p-nitrophenylhydrazone).A solution of aldehyde (8) (0.1 g) in ethanol (20 ml) was treated under reflux with the respective hydroxylamine, phenyl-, *p*-nitrophenyl-, or *p*-sulphamylhydrazine and with semicarbazide (one molar proportion) and few drops of acetic acid. Each product was worked up in the usual manner and formed needles on recrystallization from ethanol (Table 1).

Imidazole pyrazole derivative (16). To a solution of 3-formyl-4,5 (1H)-pyrazolinedione-4-(*p*-nitrophenylhydrazone) (0.1 g) in methanol (20 ml) was added *o*-phenylenediamine (20 ml) and acetic acid (5 ml) and the mixture was boiled under reflux for 3 hrs. The solution was concentrated to small volume and left to cool at room temperature. The solid that separated out was filtered, washed with methanol and ether and dried (yield 60 mg). On recrystallization from methanol it gave yellow needles, m.p. 192-194° υ_{max} KBr 3100 (NH) and 1680 cm^{-1} (OCN).

Analysis: Calc. for $C_{16}H_{13}N_7O_3$: C, 54.70; H, 3.70; N, 27.92. Found: C, 54.77; H, 3.99; N, 27.71.

D-erythro-2,3-hexodiulosono-1,4-lactone 2-(p-nitrophenylhydrazone (18). A solution of dehydro-D isoascorbic acid obtained by oxidising an aqueous solution of isoascorbic acid (10 g) with iodine (13 g) in ethanol (50 ml), was treated with *p*-nitrophenylhydrazine (0.8 g) and few drops of acetic acid. After keeping at room temperature for 3 days. D-erythro-2,3-hexodiulosono-1,4-lactone 2-(*p*-nitrophenylhydrazone) crystallized out, which was filtered, washed successively with water, ethanol and dried (yield 1 g). On recrystallization from ethanol it gave yellow needles, m.p. 178-179°; *m/z* 309(70), 291(36), 248(80), 127(32), 163(27), 150(52) 123(100). υ_{max} KBr 3450 (OH), 1720 (lactone C=O), and 1680 cm-1 (C=O);

Analysis: Calc. for $C_{12}H_{11}N_3O_7$: C, 46.60; H, 3.56; N, 13.59. Found: C, 46.19; H, 3.32; N, 13.74.

D-erythro-2, 3-hexodiulosono-1, 4-lactone 2-(p-nitrophenylhydrazone) 3-p-sulphamylphenylhydrazone) (19). To a solution of D-erythro-2, 3-hexodiulosono-1, 4-lactone 2-(*p*-nitrophenylhydrazone) (1 g) in ethnol (50 ml) was added *p*-sulphamyl phenylhydrazine (1 g) and acetic (5 ml) and the mixture was boiled under reflux for 4 hrs. It was concentrated to a small volume and left to cool at room temperaure. The solid that separated out was filtered, washed with ethanol and ether and dried (yield 1 g). On recrystallization from dioxan-ethanol (1:1) mixture it gave red needles, m.p. 226-228°; υ_{max} KBr 3420 (OH), 1740 (lactone C=O), 1600 (C=N), and 1200 cm^{-1} (CS).

Analysis: Calc. for $C_{18}H_{18}N_6O_8S$: C, 45.19; H, 3.76; N, 17.57. Found: C, 45.54; H, 3.92; N, 17.38.

5,6-Di-o-acetyl-D-erythro-2, 3-hexodiulosono-1, 4-lactone 2-(p-nitrophenylhydrazone) 3-(p-sulphamylphenylhydrazone) (20). A solution of compound (19) (0.1 g) in dry pyridine (10 ml) was treated with acetic anhydride (5 ml) and kept overnight at room temperature. The mixture was poured onto crushed ice, and the product was filtered, successively washed with ethanol and ether and dried (yield 1 g). On recrystallization from ethanol it formed red needles, m.p. 165-167° υ_{max} KBr 1740 cm^{-1} (ester and lactone C=O).

Analysis: Calc. for $C_{22}H_{22}N_6O_{10}S$: C, 46.97; H, 3.91; N, 14.94. Found: C, 46.71; H, 3.62; N, 14.82.

1-p-Sulphamylphenyl-3-(D-erythro-glycerol-1-yl)-4, 5-pyrazolinedione 4-(p-nitrophenylhydrazone) (21). A solution of compound (19) (1 g) in ethanol (20 ml) was treated with hydrazine hydrate (2 ml) and boiled under reflux for 1 hr, acidified with acetic acid and left to cool at room temperature. The solid was filtered, successively washed with water, ethanol and ether and dried (yield 1 g). On recrystallization from ethanol it gave orange needles m.p. 192-193°; υ_{max} KBr 3420 (CH), 1680 (OCN), and 1200 cm^{-1} (CS).

Analysis: Calc. for $C_{18}H_{18}N_6O_8S$: C, 45.19; H, 3.76; N, 17.57. Found: C, 45.32; H, 3.57; N, 17.30.

1-p-Sulphamylphenyl-3-(tri-o-acetyl-D-erythro-glycerol-1-yl) 4,5-pyrazolinedione-4-(p-nitrophenylhydrazone) (22). A solution of compound *(21)* (0.1 g) in pyridine (10ml) treated with acetic anhydride (5 ml) and kept overnight at room temperature. The reaction mixture was poured onto crushed ice, and the product was filtered, successively washed with water, ethanol and ether and dried (yield 70 mg). Compound (22) on recrystallization from ethanol gave orange needles, m.p. 211-213° υ_{max} KBr 1740 (ester), 1680 (OCN) 1600 (C=N), and 1200 cm^{-1} (CS). ^{1}H-nmr data (DMSO-d): δ2.0, 2.04, 2.12 (3s, 9H, 3 $OCOCH_3$), 4.32 (m, 2 H, H-3') 5.80 (m, 1 H, H-2'), 6.20 (d, 1H, H-1'), 7.32-8.3 (m, 10 H, aromatic protons + NH_2), 13.28 (s, 1 H-1') 7.32-8.3 (m, 10 H, aromatic protons + NH_2), 13.28 (s, 1 H, NH).

Analysis: Calc. for $C_{24}H_{24}N_6O_{11}S$: C, 47.68; H, 3.47; N, 13.90. Found: C, 47.39; H, 3.72; N, 13.74.

3,6-Anhydro-3-(p-sulphamylphenylazo)-D-erythro-2-hexulosono-1, 4-lactone-2-(p-nitrophenylhydrazone) (23) . To a solution of compound (19) (1 g) in ethanol (50 ml) cupric chloride (1 g) was added, and the mixture was boiled under reflux for 1 hr. The solution was concentrated to a small volume. Hot water (20 ml) was added to the concentrated filtrate and left to cool at room temperature. The solid that separated out was filtered, washed with water and ethanol and dried (yield 0.5 g). On recrystallization from ethanol it formed yellow needles, m.p. 244-249°; υ_{max} KBr3400 (OH), 1730 cm^{-1} (lactone C=O).

Analysis: Calc. for $C_{18}H_{16}N_6O_8S$: C, 45.37; H, 3.36; N. 17.64. Found: C, 45.88; H, 3.82; N, 17.84.

5-o-Acetyl-3,6-anhydro-3-(p-sulphamylphenylazo)-D-erythro-2-hexulosono-1,4-lactone-2-(p-nitrophenylhydrazone (24). A solution of compound (23) (0.1 g) in dry pyridine (10 ml) was treated with acetic anhydride (10 ml), and kept overnight at room temparature. The mixture was poured onto crushed ice, and the product that separated was filtered, successively washed with water, ethanol and ether and dried (yield 60 mg). On recrystallization from ethanol it formed yellow needles, m.p. 154-155°; υ_{max} KBr1730 cm^{-1} (lactone C=O and ester. ^{1}H-nmr data ($CDCl_3$): δ 2.0 (s, 3H, $OCOCH_3$), 4.32 (m, 2H, H-6), 5.30 (m, 1H, H-5), 5.58 (d, 1H, H-4) 7.30-8.32 (m, 10H, aromatic protons + NH_2), 12.28 (bs, 1H, NH).

Analysis: Calc. for $C_{20}H_{18},N_6O_9S$: C, 46.33; H, 3.47; N, 16.12. Found: C, 46.52; H, 3.32; N, 16.43.

5-o-Benzoyl-3,6-anhydro-3-(p-sulphamylphenylazo)-D-erythro-2-hexulosono-1,4-lactone-2-(p-nitrophenylhydrazone) (25). A solution of compound (23) (0.1 g) in dry pyridine (10 ml) was treated with benzoyl chloride (1.0 ml), and kept overnight at room temperature. The mixture was poured onto crushed ice, and the solid that separated was filtered, washed with water and ethanol and dried (yield 49 mg). On recrystallization from ethanol it formed yellow needles, m.p. 220-222°; υ_{max} KBr1750 cm^{-1} (lactone C=O and ester).

Analysis: Calc. for $C_{25}H_{20}N_6O_9S$: C, 51.72; H, 3.45; N, 14.48. Found: C, 51.47; H, 3.45; N, 14.68.

D-erythro-2-hexulosono-1,4-lactone-2-(p-nitrophenylhydrazone)-3-oxime (26). A solution of the monohydrazone (18) (1 g) in ethanol (50 ml) was treated with hydroxylamine hydrochloride (1 g), sodium acetate (1 g), and few drops of acetic acid, and boiled under reflux for 3 hr. It was concentrated, and hot water (20 ml) was added to it and left to cool at room temperature. The solid was filtered, successively washed with water, ethanol and ether and dried (yield 0.8 g). On recrystallization from ethanol it formed yellow needles, m.p. 238-240°; υ_{max} KBr3400 (OH), 1730 (lactone C=O), and 1600 cm^{-1} (C=N).

Analysis: Calc. for $C_{12}H_{12}N_4O_7$: C, 44.44; H, 3.70; N, 17.28. Found: C, 44.33; H, 3.62; N, 17.22.

4-(2,3-Di-o-acetyl-D-erythro-glycerol-1-yl)-2-p-nitrophenyl- 1,2, 3-triazole-5-carboxylic acid-5, 1'-lactone (27). A suspension of compound (26) (0.1 g) in dry pyridine (10 ml) was treated with acetic anhydride (10 ml) and kept overnight at room temperature. The mixture was poured onto crushed ice, and the product that separated out was filtered., washed with water, ethanol and dried (yield 60 mg). Compound (27) crystallized from ethanol in colourless needles, m.p. 149-151°; υ_{max} KBr1800 (lactone C=O), 1740 (ester), and 1600 cm^{-1} (C=N); ^{1}H-nmr data ($CDCl_3$); δ2.08, 2.14 (2s, 6H, 2 $OCOCH_3$). 4.45 (m, 2 H, H-3'), 5.59 (q, 1 H, H-2'). 5.94 (d, 1H,

H-1'), 8.12-8.26 (m, 4 H, aromatic protons); *m/z* 390(54), 389(74), 347(82), 331(26), 295(88), 245(39), 239(63), 211(26), 191(28), 169(100), 150(78) 136(62), 122(14) 69(36) 57(28), 43(14), 41(68).

Analysis: Calc. for $C_{16}H_{14}N_4O_8$: C, 49.23; H, 3.58; N, 14.35. Found: C, 49.52; H, 3.74; N, 14.22.

Biological activity. Compounds 4, 22, 24 and 27 were tested against eleven different microorganisms. These microorganisms belong to bacteria (*Bacillus subtillis*), three species of *Thermophilic bacillus*, *Escherichia coli*, and *Pseudomonas* sp), *Actinomecetes* sp., and *Thermoactinomyces vulgaris*), and Fungi (*Aspergillus fumigatus*, *Penicillium* sp and *Helminthosporium* sp). They were isolated from soils of different localities of the Eastrn region of Saudi Arabia.

Diffusion plate method was used for this test. A volume of 0.1 ml of diluted 16 hrs cultures in case of bacteria and actinomycetes, and 0.1 ml of spore suspension in case of fungi were spread on separate agar plates of glucose-yeast extract-agar medium. Three porous cups were made in each plate using sterile cork-borer (8 mm in diamaetr). In each pore 0.15 ml of the solution (50 mg/ml of absolute ethanol) to be tested was placed. The plates were incubated at suitable temperature for each organism (30° for Mesophiles and 55° for Thermophiles) for 24 hrs in case of Bacteria and Actinomycetes, and for 4 days in case of Fungi. Triplicates were made for each microorganism. After incubation period mean diamters of the inhibition zones were measured to the nearest 0.5 mm (Table 2).

Results and Discussion

Treatment of dehydro-L-ascorbic acid 2-(*p*-nitrophenylhydrazone (1) (L-threo-2, 3-hexodiulosono-1, 4-lactone 2-(*p*-nitrophenylhydrazone) with one equivalent of semicarbazide hydrochloride, gave L-threo-2, 3-hexodiulosono-1, 4-lactone-2-(*p*-nitrophenylhydrazone) 3-semicarbazone (2). Boiling of compound (2) with concentrated ammonia, afforded 3-(L-threoglycerol-1-yl)-4, 5(1H)-pyrazolinedione 4-(*p*-nitrophenylhydrazone) (4) on the basis of elemental analysis, infrared and mass spectral data. Its elemental analysis agreed with the molecular formula $C_{12}H_{13}N_5O_6$ and its infrared spectrum showed a carbonyl absorption band at 1680 cm^{-1} in addition to the hydroxyl absorption at 3450 cm^{-1}. The mass spectrum of compound (4) showed a molecular ion-peak at *m/z* 323, this was followed by a series of ions arising from elimination processes involving the sugar moiety and the heterocyclic ring.

Acetylation of compound (4) with boiling acetic anhydride, afforded 1-acetyl-3-(*tri-o*-acetyl-L-threo-glycerol-1-yl)-4,5-pyrazolinedion-4-(*p*-nitrophenylhydrazone) (5). The infrared spectrum of (5) showed a carbonyl absorpion at 1680 cm^{-1}, in addition to an ester band at 1740 cm^{-1}. ^{1}H-nmr spectrum of compound (5) showed three acetyl group signals at δ 2.06, 2.08 and 2.20, in addition to an N-acetyl group signal at δ 2.58.

Treatment of L-threo-2, 3-hexodiulosono-1, 4-lactone 2-(*p*-nitrophenylhydrazone) (1) with hydrazine hydrate in boiling methanol, afforded 3-(L-threo-glycerol-1-yl)-4, 5 (1H)-pyrazolinedione 4-(*p*-nitrophenylhydrazone) (4), identical with the compound obtained on boiling L-threo-2, 3-hexodiulosono-1,4-lactone 2-(*p*-nitrophenylhydrazone) 3-semicarbazone (2) with concentrated ammonia. This indicates that hydrazine hydrate reacts more readily with the open chain monohydrazone (6) giving the intermediate mixed bishydrazone (7), that rapidly cyclizes to the pyrazole derivative (4).

Periodate oxidation of one mol of compound (4) resulted in the consumption of two moles of the oxidant with the formation of 3-formyl-4, 5 (1H)-pyrazolinedione 4-(*p*-nitrophenylhydrazone) (8). The i.r. spectrum of (8) showed a broad band at 1700 cm^{-1} (OCN and CHO). Reduction of compound (8) with sodium borohydride, afforded 3-hydroxymethyl)-4, 5-(1H)-pyrazolinedione 4-(*p*-nitrophenylhydrazone) (9). Acetylation of compound (9) with boiling acetic anhydride, gave 3-(acetoxymethyl)-1-acetyl-4, 5-pyrazolinedione-4-(*p*-nitrophenylhydrazone) (10). The ir spectrum of (10) showed an ester band at 1750 cm^{-1}, in addition to an amide band at 1680 cm^{-1}, and the ^{1}H-nmr spectrum showed two singlets, each of three proton intensity; the first at δ2.05 and was attributed to the 3-(acetoxymethyl) group, and the second at δ 2.58 was due to the acetyl group at N^{-1}. The spectrum also showed at singlet of two proton intensity at δ5.0 (CH_2), and the aromatic protons appeared as a multiplet at δ7.28-8.12, the imino-proton of the hydrazone residue at C-4 appeaed at δ13.4.

Condensation of compound (8) with hydroxylamine, 3-formyl-4,5 (1H)-pyrazolinedione 3-(*p*-nitrophenylhydrazone) 3-oxime (11) was obtained, similarly, when the aldehyde (8) was condensed with phenyl-, *p*-nitrophenyl-, and *p*-sulphamylphenylhydrazine, it yielded the corresponding hydrazones (12-14) (Table 1). Also, it was condensed with semicarbazide to give compound (15). The pyrazole aldehyde (8) reacts also with *o*-phenylenediamine to give (16) (Table I).

Unimolecular condensation of dehydro-D-isoascorbic acid (17) (D-erythro-2, 3-hexodiulosono-1, 4-lactone) with *p*-nitrophenylhydrazine, at room temperature, condensation occurs mainly, with the more active C-2 carbonyl group to give the monohydrazone (18) (D-erythro-2, 3-hexodiulosono-1,4-lactone 2-(*p*-nitrophenylhydrazone). Its i.r. spectrum showed the lactone carbonyl group at 1720 cm^{-1}, and the hydroxyl absorption at 3450 cm^{-1}, in addition to a carbonyl absorption (C-3) at 1680 cm^{-1}. Treatment of compound (18)

with *p*-sulphamylphenylhydrazine, afforded the red mixed bishydrazone (19), whose infrared spectrum showed the lactone carbonyl group at 1740 cm^{-1} and the hydroxyl absorption at 3420 cm^{-1}. Acetylation of compound (19) with boiling acetic anhydride, gave the di-*o*-acetyl derivative (20).

Treatment of the bishydrazone (19) with hydrazine hydrate in boiling methanol, afforded 1-*p*-sulphamylphenyl-3-(D-erythro-glycerol-1-yl)-4, 5-pyrazolinedione 4-(*p*-nitrophenylhydrazone) (21). Its i.r. spectrum showed carbonyl absorption at 1680 cm^{-1} (OCN), in addition to hydroxyl band at 3420 cm^{-1}. Acetylation of compound (21) with boiling acetic anhydride, gave 1-*p*-sulphamylphenyl-3-*(tri-o*-acetyl-D-erythro-glycerol-1-yl)-4, 5- pyrazoledione 4- (*p*-nitrophenylhydrazone (22).

Oxidation of D-erythro-2, 3-hexodiulosono-1, 4-lactone 2-(*p*-nitrophenylhydrazone) 3-(*p*-sulphamylphenylhydrazone) (19) with cupric chloride in ethanolic solution, gave the bicyclic azo compound (23) namely, 3, 6-anhydro-3-(*p*-

TABLE 1. SOME PROPERTIES OF COMPOUNDS 11-15.

Compd. No.	R	Colour	M.p. °C	Molecular formula		Analysis C	H	N	υ (cm^{-1}) OH	NH	OCN
11	OH	Orange	>270	$C_{10}H_8N_6O_4$	Requires	43.62	2.89	30.43	3450	3020	1680
					Found	43.62	3.21	30.09			
12	NHC_6H_5	Red	>270	$C_{16}H_{13}N_7O_3$	Requires	54.70	3.70	27.92	-	3100	1670
					Found	54.92	3.62	28.25			
13	$NHC_6H_4NO_2$-*p*	Orange	142-144	$C_{16}H_{12}N_8O_5$	Requires	48.48	3.03	28.28	-	3000	1680
					Found	48.20	3.23	28.51			
14	$NHC_6H_4SO_2NH_2$-*p*	Red	209-211	$C_{16}H_{14}N_8O_5S$	Requires	44.65	3.26	26.04	-	3100	1675
					Found	44.37	3.02	26.36			
15	NH-CO-NH_2	Red	262-263	$C_{11}H_{10}N_8O_4$	Requires	41.51	3.14	35.22	-	3080	1680
					Founnd	41.93	3.42	35.37			

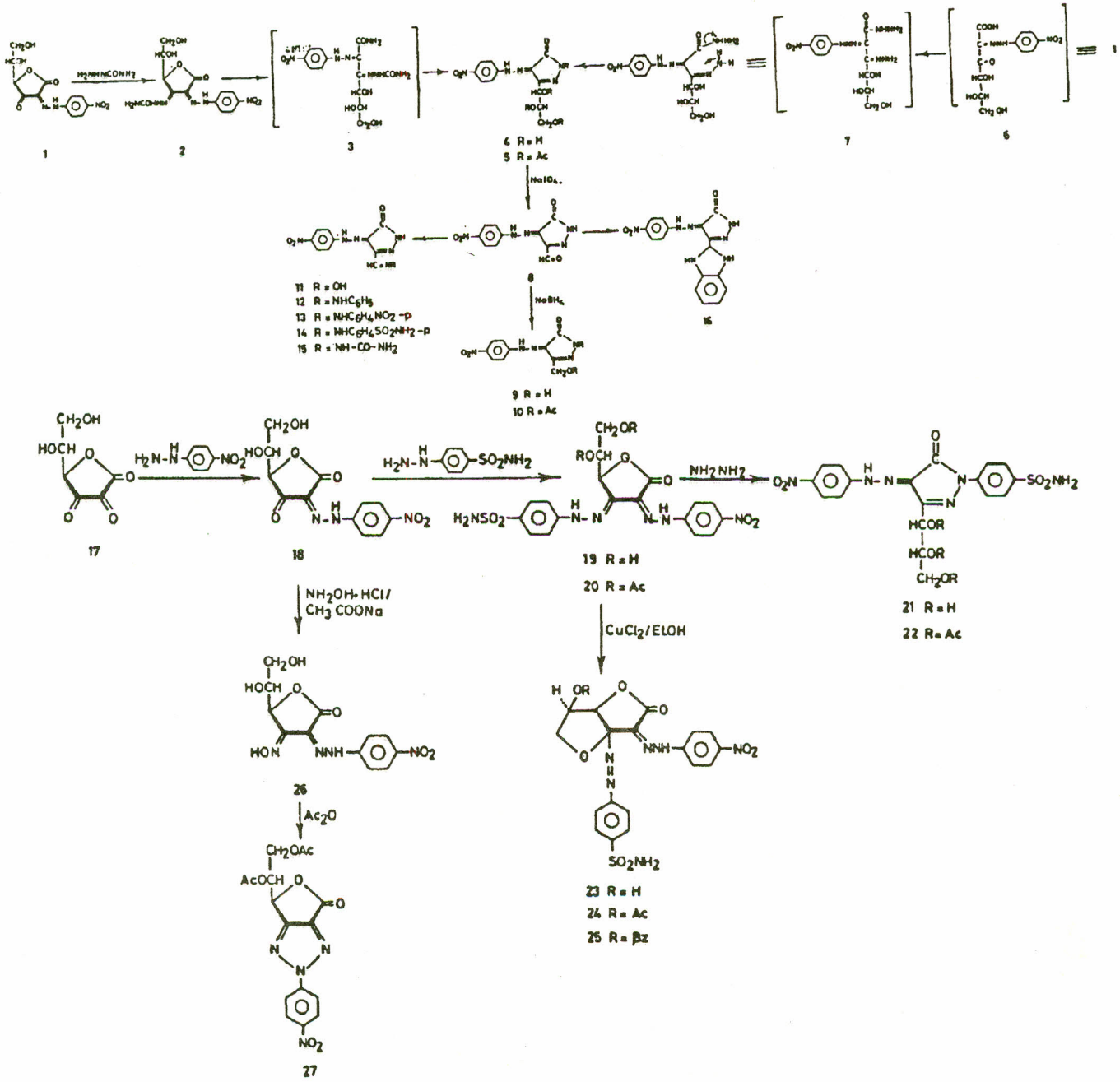
TABLE 2. ANTIMICROBIAL EFFECT OF COMPOUNDS 4, 22, 24, AND 27.

Microbial strain	Compound 4 Antimicrobial effect	Compound 4 Inhibition zone mean diameters (mm)	Compound 22 Antimicrobial effect	Compound 22 Inhibition zone mean diameters (mm)	Compound 24 Antimicrobial effect	Compound 24 Inhibition zone mean diameters (mm)	Compound 27 Antimicrobial effect	Compound 27 Inhibition zone mean diameters (mm)
BACTERIA:								
Bacillus subtilis	+	15 ± 0.0	++	23 ± 0.0	+	14 ± 0.0	++	20 ± 0.0
Thermophilic Bacillus sp_1	++	35 ± 0.0	++	25 ± 0.0	–	–	++	20 ± 0.0
Thermophilic Bacillus sp_2	++	35 ± 0.0	–	–	–	–	++	–
Bacillus sp_3	+	10 ± 0.0	–	–	++	15 ± 0.0	++	17 ± 0.0
Escherichia coli	++	18 ± 0.0	–	–	++	16 ± 0.0	++	17 ± 0.0
Pseudomonas sp	++	25 ± 0.0	++	30 ± 0.0	++	15 ± 0.0	++	17 ± 0.0
Micrococcus sp								
ACTINOMYCETES:								
Streptomyces sp	++	18 ± 0.0		25 ± 0.0	++	20 ± 0.0	++	15 ± 0.0
Thermoactinomyces Vulgaris	++	25 ± 0.0		15 ± 0.0	–	–	**	**
FUNGAI:								
Aspergillus fumgatus	++	19.5 ± 0.4		25 ± 0.0	++	20 ± 0.0	++	19.5 ± 0.3
Aspergillus flavus					++	20.5 ± 0.0	–	–
Aspergillus niger					–	15 –	–	–
Penicillium sp	++	26.5 ± 0.3		20 + 0.0	++	*15.5 ± 0.4	**	**
Helminthosporium sp	++	24 ± 0.0		28 ± 0.0	++	15.5 ± 0.0	–	–

– = not effective. + = less effective. ++ = more effective. * = then activator after one weak. ** = activator.

sulphamylphenylazo)-D-erythro-2-hexodiulosono-1, 4-lactone 2-(*p*-nitrophenylhydrazone). Its i.r. spectrum showed the lactone carbonyl group at 1720 cm^{-1} in addition to the hydroxyl band at 3400 cm^{-1}. Acetylation of compound (23) with boiling acetic anhydride, gave the monoacetyl derivative (24). Similarly, treatment of (23) with benzoyl chloride and pyridine, gave the monobenzoyl derivative (25).

On treatment of the monohydrazone (18) with hydroxylamine, D-erythro-2, 3-hexodiulosono-1, 4-lactone 2-(*p*-nitrophenylhydrazone) 3-oxime (26) was obtained. Boiling of compound (26) with acetic anhydride resulted in acetylation of the hydroxyl groups on C-5 and C-6, and elimination of a molecule of water from the hydrazone residue and the hydroxylamino group, to form 2-(p-nitrophenyl)-4-(2,3-di-o-acetyl-D-erythro-glycerol-1-yl)-1, 2,3-triazole-5-carboxylic acid-5, 1'-lactone (27). This reaction is similar to that conducted [4] on the phenyl derivative. The infrared spectrum of (27) showed the lactone carbonyl group at 1800 cm^{-1}. ^{1}H-nmr spectrum of (27) showed two distinct acetyl group signals at δ2.08, and 2.14; the C-3' methylene group appeared as a multiplet centered at δ4.45, followed by a multiplet at δ 5.59 (H-2'), and a doublet at δ5.94 (H-1'). The aromatic protons appeared as a multiplet at δ8.12- 8.26. No protons appeared in the offset region of the spectrum.



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