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SYNTHESIS OF SOME NEW NITROGENOUS DERIVATIVES OF GLYCYRRHETIC ACID WITH POTENTIAL BIOLOGICAL ACTIVITY

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The synthesis of some nitrogenous derivatives from glycyrrhetic acid hydrazides (III) by incorporating its C-30 to form 1', 3', 4'-oxadiazole (V), 4'-acetyl-5-aryl-1'-oxadiazoline (VII), 1',3',4'-triazole (X), carboxymethyl thiotriazole (XII) and 1',3',4'-thiodiazoline-5'-thion (XIII) rings are reported. The reactions of the hydrazide III with aromatic aldehydes, thiocyanates, isocyanates and its 3-acetyl derivative (XVI) with aniline and ethyl glycinate were studied. The antimicrobial activity of some derivatives was tested against some microorganisms.

Key words: Glycyrrhetic acid, Oxadiazole, Triazole, Thiodiazole derivatives.

Introduction

The natural polyfunctional pentacyclic triterpene glycyrrhetic acid and many of its nitrogenous derivatives were well known for their biological activity and/or medicinal applications. Literature are rich with numerous publications dealing with their antiulcer activity [1-4] and its clinical applications [5,6], anti-inflamatory [7-9], antihormonal [8-9] and antineoplastic [10] activity.

In the present work acetylglycyrrhetylhydrazide (III) was proposed as a potential starting material for further functionalization towards the synthesis of new derivatives containing 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,3,4-triazoles rings. These five membered heterocycles are well known to have potential biological activities. The therapeutic action may involve anti-inflamatory [13], antibacterial [14,15], anticonvulsant [16] and hypontic [17,18] activity.

Experimental

Melting points are uncorrected. The infrared spectra were carried out in potassium bromide on a PU 9712 infrared spectrophotometer. The MS spectra were measured on a varian instruments, MAT CH-5, NMR spectra were measured on Varian Gemini 200 (^{1}H NMR=200 MHz and ^{13}C = 50 MHz) using deuterochloroform as a solvent. Melting points, analytical and biological test data of the prepared compounds are given in Tables 1 and 4.

Acetylglycyrrhetyl chloride (II). A solution of acetyl glycyrrhetic acid (I) (5 g, 9.8 mmol) in thionyl chloride (31.2 ml) and dimethyl formamide (3 drops) was heated under reflux for 1 hr. The excess thionyl chloride was removed in vacuo and the residue was crystallized from benzene-pet. ether (60-80°C to give 4.2 g (81%) of pale yellow crystals of II, m.p. 290-91°C (lit. m.p. 290-91°C, 72%) [19].

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Acetylglycyrrhetylhydrazide (III). A solution of II (3 g, 5.7 mmol) in dry THF or toluene (25 ml) was added dropwise over a period of 30 mins. to a stirred solution of hydrazine hydrate (1 ml, 90%) in THF (15 ml) at 0°C. After additional 30 mins. the organic layer was washed with water, dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo. The residue was crystallized from benzene-pet. ether (60-80°C) to give 2.9 g (98 %) of colourless crystas of III. The IR spectrum of III showed bands at 3400, 3342 cm^{-1} (NH str.), 1730 cm^{-3} (CH_3COO-) and 1654cm^{-1} (COCH = C$\diagdown$ -CONH).

TABLE 1.

Compd. No.	m.p. °C	Molecular formula (Molecular weight)	Analysis Cald./Found C %	H %	N %
III	289-91	$C_{32}H_{50}N_2O_4$	73.00	9.50	5.32
		(526)	72.90	9.30	5.10
IV	>350	$C_{64}H_{96}O_8N_2$	75.29	9.41	2.74
		(1020)	74.30	9.30	2.60
V	313-15	$C_{64}H_{94}O_7N_2$	76.65	9.38	2.79
		(1002)	75.90	9.20	2.50
VIa	197-99	$C_{39}H_{54}N_2O_4$	76.22	8.79	-
		(614)	76.00	8.60	-
VIb	295-97	$C_{40}H_{56}N_2O_5$	74.53	8.69	-
		(644)	74.30	8.50	-
VIc	260-61	$C_{39}H_{53}N_2O_4Cl$	72.16	8.17	-
		(648.5)	71.70	8.30	-
VId	209-10	$C_{40}H_{56}N_2O_6$	72.73	8.48	-
		(660)	72.50	8.20	-
VIe	165-67	$C_{41}H_{59}N_3O_4$	74.89	8.98	-
		(657)	74.70	8.70	-
VIf	297-98	$C_{39}H_{53}N_3O_6$	71.02	8.04	-
		(659)	70.52	8.10	-
VIIa	210-11	$C_{41}H_{56}N_2O_5$	75.00	8.54	4.27
		(656)	74.90	8.30	4.10

(Continue...)

(Table 1 continue...)

VIIb	262-63	$C_{42}H_{58}N_2O_6$	73.47	8.45	8.08
		(686)	73.30	8.30	3.80
VIIc	181-83	$C_{41}H_{55}N_2O_6Cl$	71.25	7.97	4.06
		(690.5)	71.10	7.70	3.70
VIII	250	$C_{45}H_{70}N_2O_5$	75.21	9.75	3.90
		(718)	75.00	9.50	3.70
IXa	255-57	$C_{33}H_{51}N_3O_5$	69.59	8.96	7.38
		(569)	69.30	8.60	7.10
IXb	168-70	$C_{35}H_{55}N_3O_5$	70.35	9.21	-
		(597)	70.00	9.10	-
IXc	191-93	$C_{39}H_{61}N_3O_5$	71.89	9.37	-
		(651)	71.70	9.30	-
IXd	187-89	$C_{39}H_{55}N_3O_5$	72.56	8.53	-
		(645)	72.30	8.32	-
IXe	179-80	$C_{39}H_{55}N_3O_4S$	70.80	8.32	-
		(661)	70.70	8.30	-
IXf	266-67	$C_{40}H_{57}N_3O_5S$	69.46	8.25	-
		(691)	69.30	8.10	-
Xa	197-98	$C_{33}H_{51}N_3O_3$	73.74	9.50	7.82
		(537)	73.50	9.30	7.70
XIa	218-19	$C_{38}H_{53}N_2O_3S$	72.27	8.40	6.86
		(631)	72.10	8.10	6.70

N^1, N^2-Diacetylglycyrrhetylhydrazine (IV). Method A. The above procedure was repeated but at 5°C to give 4.6 g (80%) of colourless crystals of IV.

Method B. The above procedure was repeated using acetyl-glycyrrhetylhydrazide (III), (1.9 g, 3.6 mmol) instead of hydrazine to give 3.0 g (82%) of IV. The IR spectrum of IV showed bands at 3542 cm^{-1} (NH), 1726 cm^{-1} (CH_3 COO-) and 1655 cm^{-1} (COCH=C$\diagdown$, CONH).

2',5'-Di-(30nor, 20β-acetylglycyrrhetyl)-1',3',4'-oxadiazole (V). A mixture of IV (0.5 g, 0.49 mmol) in acetic anhydride (1.25 ml) and pyridine (3 drops) was refluxed for 1 hr. Excess acetic anhydride was then removed in vacuo and the residue was crystallized from methanol to give 0.36 g (73%) of colourless crystals of V. The IR spectrum showed bands at 1735 cm^{-1} (CH_3COO-) and 1645 cm^{-1} (COCH =C$\diagdown$).

N^1-Acetylglycyrrhetyl- N^1, N^2-dicyclohexylurea (VIII). Dicyclohexylcarbodiimide (0.4g, 2mmol) was added to a stirred solution of I (1g, 0.2 mmol) in THF (10 ml) and DMF (2 drops) at 40°C. After 5 hrs, the reaction mixture was left overnight at room temperature. Few drops of water was added and the precipitate formed was filtered, washed and dried. Crystallization from methanol afforded 1.39 (93 %) of colour-less crystals of VIII. The IR spectrum showed bands at 3328 (NH), 1736 (CH_3C00-), and 1655, 1626 (COCH =C$\diagdown$, CON)cm^{-1}.

Acetylglycyrrhetylhydrazone (VIa-f). A solution of III (0.5 g, 0.95 mmol) and each of the appropriate aromatic aldehydes (1 mmol) in dry THF (5 ml) was stirred under reflux for the appropriate time. The solvent was removed in vacuo and the residue was crystallized from benzene-pet. ether (60-80°C) to give the hydrazones VIa-f respectively. The IR spectra of compounds VIa-f showed NHυ bands in the region of 3444-3562 cm^{-1} and three C=O bands in the region of 1735-1675 cm^{-1}. Table 2 illustrates the reacted aldehydes, the time of each reaction and the yield of each product.

TABLE 2.

Product No.	Reacted aldehyde	Time of reaction	Yield %
VIa	Benzaldehyde	16 mins	98
VIb	Anisaldehyde	2 hrs	98
VIc	P-chlorobenzaldehyde	2 hrs	95
VId	Vanilline	3 hrs	96
VIe	P-N, N-Dimethylamine-benzaldehyde	2 hrs	98
VIf	P-Nitrobenzaldehyde	3 hrs	96

2'-(30 Nor, 20 β-acetylglycyrrhetyl)-4'-acetyl-5'-aryl-1',3',4'-oxadiazolines (VII) a-c). A solution of each of the hydrazones VIa-c (0.8 mmol) in acetic anhydride (1.25 ml) was heated under reflux for 1 hr. The excess anhydride was removed in vacuo and the residue was crystallized from $CHCl_3$/MeOH to give the corresponding VIIa-c respectively in 85-87% yield. The IR spectra of compounds VIIa-c showed three C=O bands in the region of 1735-1675 cm^{-1}.

The MS spectrum of VIIb showed M^+ at m/z 686 (20%).

The PMR spectrum of VIIb shows signals of 7 methyl groups of the glycyrrhetic acid as singlets at 0.82, 0.87, 1.11, 1.114, 1.22, 1.34 and 1.42 ppm [20]. in addition to the signals at 2.04 (S, $\underline{CH_3}$-C-00), 2.26, (S, $\underline{CH_3}$CON), 3.91 (S, $\underline{CH_3}$O), 4.50 (q, C_3-$\underline{H}$); 5.40 (S, C_{12}-$\underline{H}$), 6.85 (S, N$\rangle$$\underline{CH}$-$C_6H_4$-), 6.91 (d, $2H_{3''}$, J=9.8Hz), 7.31 (d, $2H_{2''}$, J = 9.8 Hz) ppm [21].

^{13}C NMR signals* of VIIb are 39.81 (C_1), 28.15 (C_2), 82.50 (C_3), 38.63 (C_4), 56.83 (C_5), 19.08 (C_6), 34.43 (C_7), 44.98 (C_8) 63.56 (C_9), 38.70 (C_{10}), 202.20 (C_{11}), 130.58 (C_{12}), 171.04 (C_{13}), 47.14 (C_{14}), 25.30 (C_{15}), 25.30 (C_{16}), 33.66 (C_{17}), 49.08 (C_{18}), 40.51 (C_{19}), 42.51 (C_{20}), 32.43 (C_{21}), 38.87 (C_{22}), 29.79 (C_{23}), 18.15 (C_{24}), 14.41 (C_{25}), 20.40 (C_{26}), 23.07 (C_{27}), 30.22 (C_{28}), 30.51 (C_{29}), 164.19 ($C_{2'}$), 93.31 ($^{N}_{O}\rangle$ C-ph), 130.79 ($C_{1''}$), 129.76 ($C_{2''}$), 116.25 ($C_{3''}$), 162.30 ($C_{4''}$), 0-57.25 (OCH_3), 173.24 ($N\underline{C}OCH_3$, 25.07 (NCO-$\underline{C}H_3$), 169.92 ($O\underline{C}O\,CH_3$) and 23.07 ($OCO\underline{C}H_3$) ppm.

1-Acetylglycyrrhetylsemicarbazide (IXa). A solution of potassium cyanate (0.16 g, 1.8 mmol) in 3 ml water was added dropwise to a stirred solution of III (0.5 g, 0.95 mmol) in acetic acid (10 ml, 50%) at room temperature. After 15 min., the precipitate formed was filtered, washed and finally crystallized from diluted methanol to afford 0.46 g (85%) of colourless

TABLE 3.

Product No.	Reacted isocyanate compounds	Time of reaction	Eluted solvents used for C.C.	Yield %
IXb	Ethylisocyanate	30 mins.	Ethyl acetate-benzene (75 : 25)	97
IXc	Cyclohexyl isocyanate	30 mins.	Ethyl acetate-benzene (80 : 20)	73
IXd	Phenyl isocyanate	1 hr.	Ethyl acetate-benzene (70 : 30)	79
IXe	Phenyl thioisocyanate	1 hr.	Ethyl acetate-benzene (75 : 25)	84
IXf	P-Methyoxyphenyl thioisocynate	1hr.	Ethyl acetate-benzene (75 : 25)	95

crystals of IXa. The IR spectrum showed bands at 3418 (NH) and 1728 - 1656 cm^{-1} (CH_3 COO-, CO-CH = C$\diagdown$, CO-NH).

1-Acetylglycyrrhetylsemicarbazide (IXb-d) and 1-Acetylgly-cyrrhetylthiosemicarbazide (IXe, f). To a solution of III (0.127 g, 0.24 mmol) in THF (5 ml) or ethanol (5 ml) in case of IXe, f, the appropriate isocyanate or isothiocyanate (0.2 mmol) was added. The reaction mixture was heated under reflux for the appropriate time. The solvent was then removed in vacuo and the residue was purified on column chromatography using silica gel (60-120 mesh) to give a corresponding semicarbazide IXb-d or thiosemicarbazide IXe-f. The IR spectra of IXb-f showed the characteristic bands at 3536-3296 cm^{-1} (NHυ) and 1730 - 1655 cm^{-1} (CH_3 COO-, COCH = C$\diagdown$, CON). Table 3 illustrates the reacted isocyanates and thioisocyanates, the time of each reaction, the eluted solvents used for column chromatography and the yield of each product.

2'-(30-Nor, 20 β-glycyrrhetyl)-1',3',4'-triazoles (Xa and XIa) (keto and mercapto derivatives). To a solution of each of IXb, f (1.5 mmol) in methanol (10 ml) was added potassium hydroxide (0.174 g, 3 mmol) and the reaction mixture was heated under reflux for 2 hr. After cooling it was acidified with diluted HCl, and the formed precipitate was filtered, washed and dried. It was then purified by column chromatography using silica gel (60-120 mesh) and eluted with ethyl acetate - benzene (80 - 20) to give the corresponding Xa (89% yiel) and XIa (90% yield). The IR spectrum of Xa showed bands at 3386 cm^{-1} (-OH and NHυ), 1658 cm^{-1} (br.) (COCH = C$\diagdown$, C = N, CON). The PMR of Xa shows signals at 0.78 (s, CH_3), 0.79 (s, CH_3), 0.98 (s, CH_3), 1.11 (s, 3 CH_3), 1.2 (s, CH_3), 1.35 (s, CH_3), 3.2 (m, N-CH_3-) 5.68 (q, C_3-H), 5.71 (s, C_{12}-H), 7.6 (s, HOC_3) and 8.93 (s, NH) ppm. ^{13}C NMR [22,23] of Xa shows signals at 39.81 (C_1), 28.07 (C_2), 82.48 (C_3), 38.63 (C_4), 56.79 (C_5), 19.07 (C_6), 34.44 (C_7), 45.09 (C_8), 63.54 (C_9), 38.63 (C_{10}), 202.12 (C_{11}), 130.51 (C_{12}), 170.78 (C_{13}), 47.23 (C_{14}), 25.11 (C_{15}), 25.26 (C_{16}), 33.65 (C_{17}), 48.71 (C_{18}), 40.53 (C_{19}), 43.72 (C_{20}), 33.16 (C_{21}), 37.51 (C_{22}), 29.68 (C_{23}), 18.12 (C_{24}), 18.41 (C_{25}), 20.38 (C_{26}), 23.06 (C_{27}), 30.25 (C_{28}), 29.48 (C_{29}), 171.81 ($C_{2'}$), 173.33 ($^{N}_{N}$ > C=O), 29.94 (?) (N-CH_2-CH_3) and 26.30 (?), (N-CH_2-$\underline{C}H_3$) ppm.

The reaction of IXd and IXe with potassium hydroxide. The above procedure repeated for each of IXd and IXe. After working up, glycyrrhetic acid hydrazide (XIV) was recovered instead of the expected triazoles Xb and XIb.

2'-(30-Nor, 20 β glycyrrhetyl-1-p-methoxy-phenyl-1',3',4'-triazoles-5-yl-mercapto-acetic acid (XII). To a stirred solution of XIa (0.25 g, 0.47 mmol) in aqueous KOH solution (0.05 g, 0.89 mmol, in 5 ml water) mono-chloroacetic acid (0.05 g, 0.53 mmol) was added. The reaction mixture was heated under reflux for 3 hr. After cooling, it was acidified with diluted HCl and the formed precipitate was filtered, washed and crystallized from chloroform to give 0.17g (63%) of yellow crystals of XII. The IR spectrum of XII showed bands at 3296 (-OH), 1730 (S-CH_2-$\underline{C}$-OH) and 1657 C (COC=C$\diagdown$) cm^{-1}. Also, its PMR shows the expected signals at 0.79 (s, 2 CH_3), 1.0 (s, CH_3), 1.11 (s, 2 CH_3),1.28 (s,CH_3) , 1.39 (s, $C\underline{H}_3$), 3.1 (dd, S-$C\underline{H}_2$-COOH), 3.71 (q, C_3-H, 3.81 (s, $OC\underline{H}_3$), 5.68 (s, C_{12}-$\underline{H}$), 6.91 (d, $2\underline{H}_{3''}$) J= 8.8 Hz) and 7.41 (d, $2\underline{H}_{2''}$, J = 8.8Hz) ppm.

2-30 Nor, 20 β-glycyrrhetyl-4'-(H)-5'-thio-1',3',4'-thio-dia-zoline (XIII). To a solution of III (0.5 g, 0.95 mmol) and KOH (0.112 g, 2 mmol) in methanol (10 ml) was added carbon disulfide (0.14 ml). The reaction mixture was heated under reflux for 3 hr. cooled and acidified with diluted HCl. The precipitate formed was filtered and crystallized from chloroform-methanol to give 0.43 g (83%) of yellow crystals of XIII. The IR spectrum of XIII showed bands at 3574 (OHυ), 3160 (NHυ) and 1655 (COCH=C$\diagdown$) cm^{-1}.

Glycyrrhetic acid hydrazide (XIV). A mixture of III (0.5 g, 0.95 mmol) and alcoholic solution of NaOH (10 ml, 5% in 90% methanol) was heated under reflux for 2 hr. After cooling, it was acidified with diluted HCl and the precipitate formed

was filtered, washed and crystallized from diluted methanol to afford 0.3 g (65%) of XIV. The IR spectrum of XIV shows bands at 3442 cm^{-1} (-OH, NHυ) and 1655 cm^{-1} (COCH = C<, -CON). The MS spectrum of XIV showed M^+ at m/z 484 (15%).

Acetylglycyrrhetylazide (XV). Method A. A solution of $NaNO_2$ (0.07 g, 1.01 mmol) in water (5 ml), was added dropwise over a period of 10 min. to a stirred solution of III (0.5 g, 0.95 mmol) in acetic acid (10 ml, 5%) at 0°C. After 15 min. 10 ml of water was added and the precipitate formed was filtered, washed and dried in vacuo to give 0.49 g (96%) of a white powder of XV.

Method B. Ethyl chlorformate (0.37 ml, 4 mmol) was added dropwise over a period of 10 min. to a stirred solution of acetyl glycyrrhetic acid (I) (2 g, 3.9 mmol) and triethylamine (0.3 ml) in THF (20 ml) at 0°C. After 15 min., the formation of mixed anhydride is complete. A solution of sodium azide (0.33 g, 5 mmol) in THF/DMSO (5 ml, 1:1) was then added over a period of 15 min. to the reaction mixture. After additional 15 min., the reaction mixture was poured onto 50 ml of acetic acid and the precipitate formed was filtered, washed and dried in vacuo to give 2 g (98%) of XV. The IR spectrum of XV showed bands at 2130, 1326 N^--N^+=N and 1807 (CON_3)cm^{-1}.

3β-Acetoxy-ll-oxo-30-norolean-12-en-20β-isocyanate (XVI). A solution of XV (2 g, 3.7 mmol) in dry toluene (10 ml) was refluxed for 1 hr. The solvent was removed in vacuo and the reisidue was then crystallized from benzene/pet. ether (60-80°C) to give 1.5 g (85%) of XVI. The IR spectrum showed bands at 2252, 1383 (N = C =O), 1729 (CH_3COO) and 1644 (COCH=C<) cm^{-1}.

N^1 (3β-Acetoxy-ll-oxo-30-norolean-12-en-20β)-N^2-phenylurea (XVII). A solution of XVI (0.2 g, 0.39 mmol) and aniline (0.04 ml) in toluene (10 ml) was treated under reflux for 1 hr. The solvent was then removed in vacuo and the residue was crystallized from benzene/pet. ether (60-80°C) to give 0.2 g (83%) of XVII. The IR spectrum showed bands at 3468, 3376 (NHυ), 1731 (CH_3COO) and 1659 (COCH = C<) cm^{-1}.

N^1-(3β-acetoxy-ll-oxo-30-norolean-12-en-20β)-N^2-ethoxy-carbonylmethylurea (XVIII). To a solution of XVI (0.2 g, 0.39 mmol) in toluene (5 ml) was added ethyl glycinate hydrochloride (0.06 g, 4 mmol) and pyridine (2 drops). The reaction mixture was heated under reflux for 2 hr. cooled and the precipitate formed was filtered, washed and dried in vacuo to give 0.19 (79%) of XVIII. The IR spectrum of XVIII shows bands at 3380, 3350 (NH), 1730 (CH_3COO) and 1648 (COCH=C<) cm^{-1}. The MS spectrum shows M+ at m/z 612 (0.42%).

Antimicrobial Activities, The tested organisms. Three pathogenic bacteria (local strains) *Bacillus subtilis, Escherichia coli* and *Staphylococcus auraus):* one pathogenic yeast (local strains) *(Candida albicans)* and one pathogenic fungi (local strains) *(Asprigulus niger)* were used.

Media Composition (a) *Malt-agar medium (g/1).* Malt extract, 30.0, peptone from soymeal, 3.0 and agar-agar, 20.0. (b) *Potato-dextrose medium (g/1):* Potatoes extract, 200.0, glucose, 20.0 and agar-agar, 20.0.

Antimicrobial assay method. The sterile filter paper discs (5 mm) were saturated with each compound used in concentration of 400 r /disc. Then, the discs were placed on agar plates seeded with different test organisms. The plates were incubated at 37°C for 24 hr. and finally the zones of growth inhibition were measured in mm.

Results and Discussion

Acetylglycyrrhetyl chlorie (II) could be obtained in a more simplified route and a better yield (81%) than that previously reported method [19] from glycyrrhetic acid acetate (I) by using SO_2Cl_2 in presence of few drops DMF. Treatment of II with hydrazine hydrate was found to yield either the desired hydrazide III or the sym. diacetylglycyrrhetylhydrazine (IV) depending on the reaction conditions (Scheme I). Moreover, compound IV was readily cyclized to the corresponding oxadiazole V by heating under reflux in acetic anhydride and pyridine for 1 hr. The structure of V was infered from its correct analytical data and its IR spectrum.

Surprisingly, during attempted preparation of the hydrazide III from the reaction of acetylglycyrrhetic acid with hydrazine in the presence of N, N`-dicyclohexylcarbodiimide (DCC), N-acetylglycyrrhetyl-N, N`-dicyclohexyl urea (VIII) was isolated. The formation of VIII was also optimized by treating acetylglycyrrhetic acid with DCC in a mixture of THF and DMF at 40°C. The addition of DCC to the carboxylic acid group to form the N-acylurea which was previously reported by Mathias [24] and Elgamal *et al.* [25] during the esterification of bis nor- 5-cholenic acid. Also, the structure of VIII was infered from its correct analytical data and its IR spectrum.

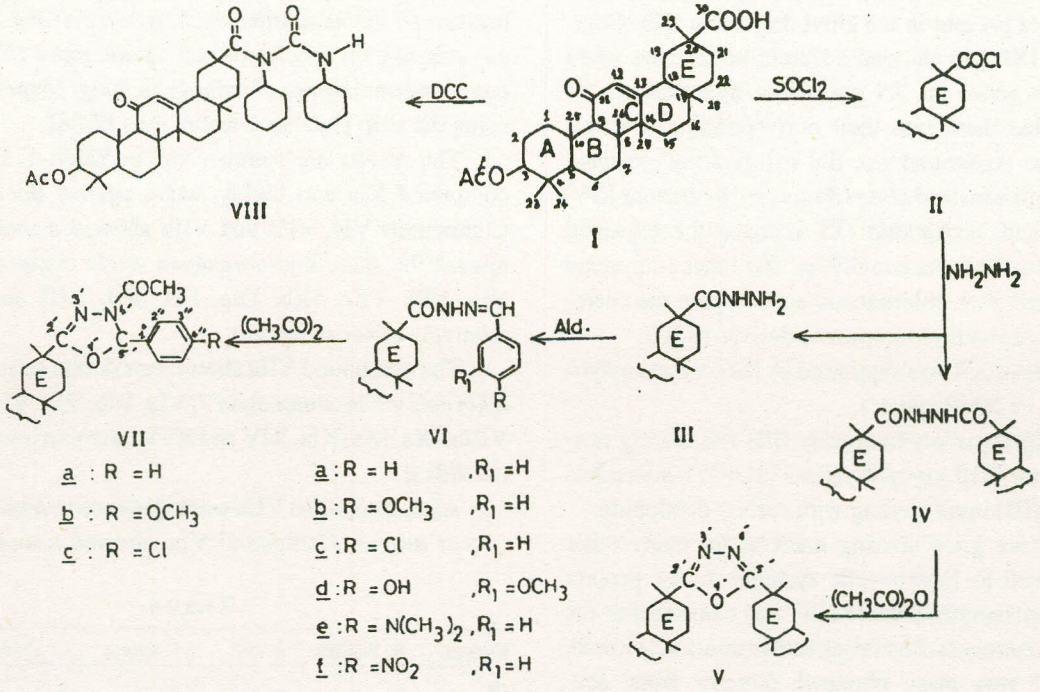
On the other hand, acetylglycyrrhetylhydrazine (III) was condensed with aromatic aldehydes, namely: benzaldehyde, anisaldehyde, p-chlorobenzaldehyde, vanillin, p-N, N-dimethylaminobenzaldehyde and p-nitrobenzaldehyde to give the corresponding hydrazones VIa-f respectively. Heating each of the hydrazones VIa-C in acetic anhydride leads to the formation of corresponding 3-acetyl-2-oxadiazolines VIIa-c respectively. The structure of the hydrazones VIa-f and oxadiazolines VIIa-c was infered from their correct analytical data.

Moreover, 2'-(30 nor, 20 β-acetylglycyrrhetyl)-4'-acetyl-5'-p-methoxy phenyl-1',3',4'-oxadiazoline (VIIb) showed the expected ^1H, ^{13}C NMR signals beside the parent molecular ion (M^+) at m/z 686 (20%).

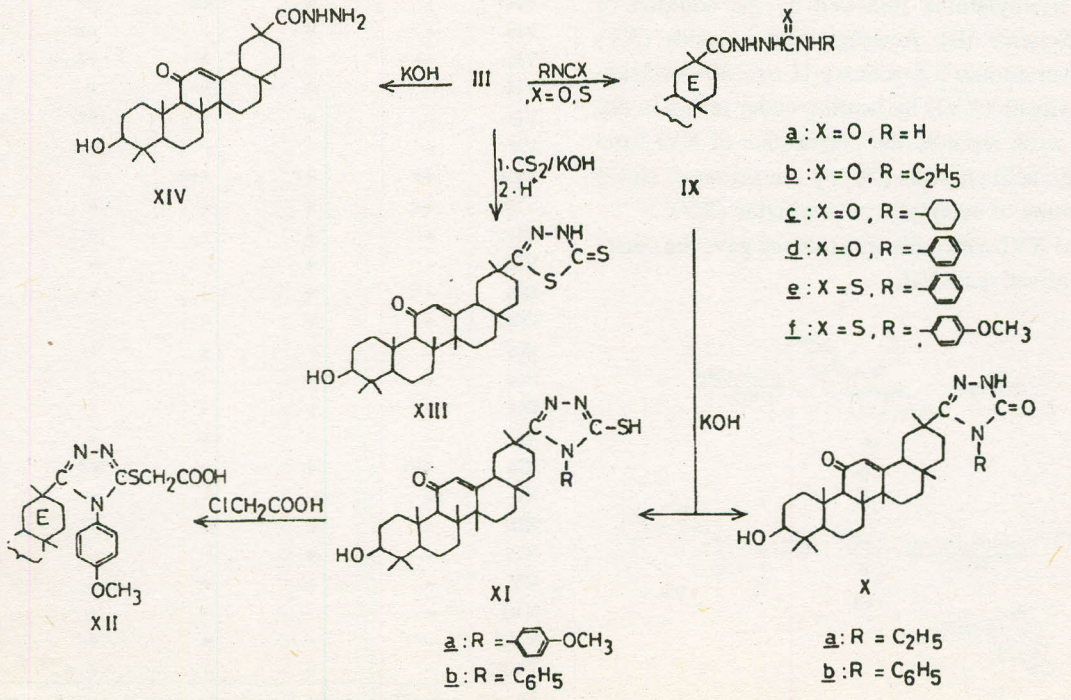
Treatment of III with potassium cyanate in dilute acetic acid or with ethylisocyanate, cyclohexylisocyanate, phenylisocyanate, phenylisothiocyanate and p-methoxy phenylisothiocyanate in dry THF leads to the foration of the corresponding acetylglycyrrhetylsemicarbazide and thiosemicarbazide derivatives IXa-f respectively (Scheme II). The structure of IXa-f was infered from their correct analytical data and their IR spectra.

Compound IX b was readily cyclized by heating under reflux in 2N potassium hydroxide to give the corresponding 1-ethyl-2-triazoline-5-one Xa.

Attempts to cyclize IXd under the same conditions to the corresponding 4'-phenyl-triazolinone derivative Xb failed and instead of its glycyrrhetic acid hydrazide (XIV) was



Scheme 1.



Scheme 2.

obtained. The latter compound was also obtained by treatment of III with 5% sodium hydroxide solution.

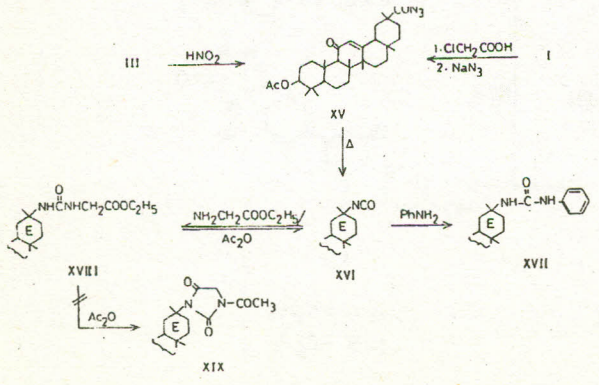
The failure of cyclization of IXd to Xb could be attributed to steric effect of both substituents on N-1` and N-4' of the semicarbazide IXd and the availability of electrons on N-4'. The latter is considerably reduced in the phenyl substituted derivative due to their involvement in the ring resonance. Such latter factor is not present in the ethyl derivative IXb. Compound IXe and IXf also showed different behaviours when subjected to the action of 2N potassium hydroxide in an attempt to cyclize them into their corresponding triazoles XIa, b. Thus, the compound IXe did not give the expected triazole XIb but give instead glycyrrhetic acid hydrazide XIV. On the other hand, compound IXf afforded the expected triazole XIa under the same conditions. The latter compound XIa was alkylated with chloroacetic acid to give the corresponding 2-carboxymethylthiotriazole derivative XII.

The structure of XII was supported by the correct analytical data, IR and its NMR spectra.

Also, acetylglycyrrhetylhydrazide (III) was readily converted to 2'-(30 nor, 20 β-glycyrrhetyl)-1',3',4'-Thiadiazoles-5'-(4H) thion (XIII) upon heating with carbon disulphide.

Isocyanates are good starting material for many other derivatives as well as heterocyclic systems. In the present study acetylglycyrrhetylhydrazide (III) was converted to the azide XV upon treatment with nitrous acid in dilute acetic acid. The azide XV was also, obtained directly from acetylglycyrrhetic acid (I) by treatment with ethyl chloroformate in presence of triethylamine followed by the addition of sodium azide (Scheme III). Acetylglycyrrhetylazide (XV) was readily converted into 3 β-acetoxy-11-oxo-30-norolean-12-en-20 β-isocyanate (XVI) by heating under reflux in dry toluene. Earlier work reported the preparation of XVI from acetylglycyrrhetic acid chloride [26] by the action of NaN3 without the isolation of acetylglycyrrhetylazide (XV).

Treatment of XVI with aniline in toluene gave the corresponding urea derivatives XVII.



Scheme 3.

Similarly treatment of XVI with ethylglycinate gave the corresponding ethoxycarbonyl methylurea derivative XVIII. Attempted cyclization of the latter to the corresponding substituted hydantion derivative XIX by heating in acetic anhydride gave instead the starting isocyanate XVI.

The antimicrobial activity of the prepared nitrogenous derivatives were tested against three strains of pathogenic bacteria *(Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus),* on species of pathogenic yeast *(Candida albicans)* and one species of pathogenic fungi *(Aspergillus niger)* using the strip plate-agar technique [27-28].

The results are summerized in Table-4. It shows that compound XIa was highly active against *Bacillus subtilis.* Compounds VIc, VIIa and VIIb showed a moderate action against the same microorganism while compounds III, IV, VIa, VIb, VIIc, IXb, IXe, IXf, XII, XIII and XVI have relatively lower sensitivity.

The compound VIIa shows a moderate action of *Escherichia coli* while compound IV, VIa, VIb, VId, VIe, VIIb, VIIc, VIIIa, IXa, IXe, XIa, XIV and XVIII shows a lower sensitivity towards it.

Also, compound VIIa was highly active against *Staphylococcus aureus.* Compound VIa, showed a moderate action

TABLE 4.

Compd.	B. Subtilis	E. coli	S. auraus	C. albicans	A. niger*
III	+	–	+	+	+
IV	+	+	–	+	+
VIa	+	+	++	++	–
VIb	+	+	+	++	–
VIc	++	–	+	++	–
VId	–	+	+	+	+
VIe	–	+	+	++	–
VIf	–	–	–	+	–
VIIa	++	++	+++	+	+
VIIb	++	+	–	+	+
VIIc	+	+	–	+	+
VIII	–	+	–	–	–
IXa	–	+	–	+	–
IXb	+	–	–	–	+
IXd	–	–	+	+	+
IXe	+	+	–	+	–
IXf	+	–	–	–	–
Xa	–	–	+	+	+
XIa	+++	+	–	++	+
XII	+	–	+	+	–
XIII	+	–	+	+	–
XIV	–	+	–	–	–
XV	–	–	+	–	+
XVI	+	–	+	+	+
XVII	–	–	+	+	+
XVIII	–	+	+	+	–

**B. sub. = Bacillus subtilis; E. coli = Escherichia coli; S. auraus = Staphylococcus auraus; C. albi. = Candida albicons; A. niger = Aspergillus niger.*

against the same microorganism while III, VIb, VIc, VId, VIe, IXd, Xa XII, XIII, XV, XVI, XVII and XVIII shows a lower sensitivity towards it.

Compounds VIa, VIb, VIc, VIe and XIa showed a moderate action against *Candida albicans* while compounds III, IV, VId, VIf, VIIa, VIIb, VIIc, IXa, IXd, IXe, Xa, XII, XIII, XVI, XVII and XVIII have relatively lower sensitivety.

Compounds III, IV, VIc, VIIa, VIIb, VIIc, IXb, IXd, Xa, XIa, XV, XVI and XVII have lower sensitivity against *Aspergillus niger.*

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