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C2C 1253 1672 215 220 22X 22Y 247 253 25Y 281 29X 29Y 30Y 311 313 314 315 31Y 321 32Y 332 337 338 339 342 34Y 351 353 355 35Y 360 361 362 363 364 365 366 368 36Y 395 39Y 440 449 579 601 60Y 620 623 624 628 62X 634 643 645 652 662 665 668 670 672 679 67X 695 790 KF KN KP LM RD

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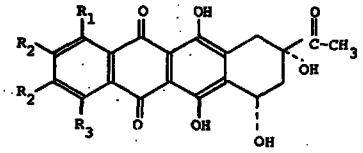
(54) ANTHRACYCLINE GLYCOSIDES

(71) We, SOCIETA FARMACEUTICI ITALIA S.p.A., a body Corporate organised and existing under the laws of Italy, of 1/2 Largo Guido Donegani-1 20121 Milan, Italy, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to new antitumour glycosides belonging to the anthracycline series and to a process of preparing them.

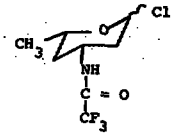
The terms daunomycin and adriamycin, which are our Trade Marks, are used herein respectively to mean daunorubicin and doxorubicin.

The new glycosides of the invention comprise condensation products of tetracyclic aglycones having a hydroxy-anthraquinone chromophoric system of the formula:



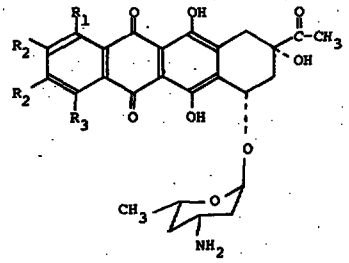
I

in which, when $R_1 = R_3 = H$, each R_2 is a hydrogen atom, a methyl or methoxy group, or a chlorine or bromine atom; when $R_1=R_2=H$, R_3 is an alkoxy group having from 1 to 4 carbon atoms; or when $R_2 = H$, R_1 and R_3 are each a methyl or methoxy group or a chlorine or bromine atom, with the halo sugar 2,3,4,6-tetradeoxy-3-trifluoroacetamido-L-threohexopyranosyl chloride:



II

After removal of the protecting group under alkaline conditions, a final product is obtained:



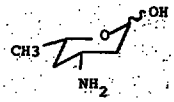
IV

where R_1, R_2, R_3 have the meanings given above.

The invention also provides adriamycin analogues of the glycosides of the general formula IV and a process for preparing the said adriamycin analogues, which process comprises converting a compound of the general formula IV into its adriamycin derivative by bromination and hydrolysis of the resulting 14-bromo-derivative.

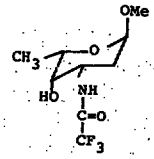
The condensation can be carried out in an organic solvent such as chloroform, methylene dichloride, diethyl ether, tetrahydrofuran, acetonitrile, or dimethylformamide in the presence of a soluble silver salt, such as silver trifluoro-methanesulphonate, as catalyst. A dehydrating agent such as a molecular sieve should preferably be present in the reaction mixture.

The halo sugar used in the condensation is a derivative of the previously unknown amino sugar 3-amino-2,3,4,6-tetradeoxy-L-threohexose:



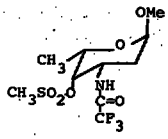
V

The halo sugar may be obtained from methyl N-trifluoroacetyl-daunosaminide:



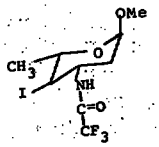
VI

This compound is reacted in dry pyridine with methane-sulphonyl chloride and so converted in a quantitative yield to its 4-O-mesyl-derivative:



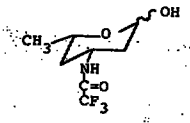
VII

By treatment with sodium iodide in methyl ethyl ketone this gives the 4-iodo-derivative:



VIII

Catalytic hydrogenation of compound VIII, followed by acid hydrolysis of the resulting methyl glycoside, affords the 2,3,4,6-tetradeoxy-3-trifluoroacetamido-L-threo-hexopyranose:



IX

Treatment of this intermediate with *p*-nitrobenzoyl chloride in dry pyridine gives the corresponding 1-*p*-nitrobenzoate. This can be treated at 0°C with dry hydrogen chloride in anhydrous methylene dichloride to obtain the desired 2,3,4,6-tetra-deoxy-3-trifluoroacetamido-L-threo-hexopyranosyl chloride II. The parent amino sugar may be obtained by alkaline hydrolysis of the intermediate IX.

When in the aglycone of structure I, $R_1 = R_2 = H$ and R_3 is methoxy, 4'-deoxy-daunomycin is finally obtained and isolated at its hydrochloride. Subsequent treatment in accordance with our British Patent Specification No. 1,1217,133 yields 4'-deoxy-adriamycin which may also be isolated as its hydrochloride. 4'-

Deoxy-daunomycin and 4'-deoxy-adriamycin each display antimitotic activity and are useful therapeutic agents for the treatment of tumour diseases.

Preparation of the intermediate 2,3,4,6-tetradeoxy-3-trifluoroacetamido-L-threo-hexopyranosyl chloride (II)

5.2 g of methyl N-trifluoroacetyl-daunosaminide VI, dissolved in 40 ml of anhydrous pyridine, are treated at −5°C with 8 ml of methanesulphonyl chloride. The reaction mixture is kept at 4°C overnight and then evaporated to dryness under vacuum. The residue is dissolved in 300 ml of chloroform and washed with water until neutrality. The organic solution is dried over anhydrous sodium sulphate and evaporated, and afforded 4.2 g of crystalline 4-O-mesyl-derivative VII: m.p. 169°—170°C; $[\alpha]_D$ −160° (c = 0.1 in $CHCl_3$); m/e 304 ($M-OCH_3$); PMR ($CDCl_3$—DMSO—d_6 4:1) : 1.26δ (d, J=6.5 Hz, CH_3—C—5), 3.18δ (s, CH_3OSO_2), 3.38δ (s, CH_3O), 4.80δ (broad s, W_H = 5.5 Hz, C—1H).

8.7 g of dried compound VII are dissolved in 150 ml of methyl ethyl ketone, treated with 5 g of sodium iodide and heated at reflux temperature for 24 h. The reaction mixture is evaporated to dryness, and the residue is suspended in 200 ml of chloroform. The insoluble material is filtered off, and the organic solution is washed with 3% aqueous sodium thiosulphate and then with water, and evaporated to dryness under vacuum. The crude product is purified by chromatography on a column of silicic acid using benzene : acetone (20:1) as eluent. There is obtained 2.3 g of crystalline compound VIII: m.p. 194—5°C; $[\alpha]_D$—100° (c = 0.1 in $CHCl_3$); m/e 367 (M^+); PMR ($CDCl_3$—DMSO—d_6 2:1) : 1.46δ (d, J=6.0 Hz, CH_3l—C—5), 3.38δ (s, CH_3O) and 4.38δ (dd, J=2.5 Hz, C—1 H).

2.3 g of compound VIII is dissolved in 80 ml of methanol and hydrogenated under pressure (15 atm) at room temperautre for 62 hours in the presence of 4 g of barium carbonate and 4 g of 10% palladium on charcoal. The catalyst is filtered off and the organic solution evaporated to dryness. The residue (1 g) is suspended in 100 ml of chloroform, the insoluble material is filtered off, and the solution is evaporated. The resulting residue is dissolved in 20 ml of water and 5 ml of glacial acetic acid is added. The solution is heated for 3 h at 90°C to evaporate the solvents and give crystalline compound IX in quantitative yield: m.p. 159—160°C; at equilibrium $[\alpha]_D^{20}$ = −80° (c = 0.1 in $CHCl_3$); m/e 210 (M—OH); PMR (DMSO—d_6): 1.03δ (d, J=6.0 Hz, CH_3—C—5 α anomer), 1.09δ (d, J=6.0 Hz, CH_3—C—5 β anomer), 1.2—1.9δ (m, C—$2H_2$ e C—$4H_2$), 4.60δ (dd, J=2.0 Hz, J'=9 Hz, C—1 Hax), 5.17δ (broad s, w_H 6Hz, C—1 Heq.).

To the solution of 1 g of compound IX in 10 ml of anhydrous pyridine, 2 g of *p*-nitrobenzoyl chloride are added. After 12 hrs at room temperature, the reaction mixture is poured onto ice and the precipitate is washed with water until neutrality. The resulting crude 2-*p*-nitrobenzoate (mixture of α and β anomer) is dried on phosphorus pentoxide for several hours under vacuum, and then dissolved in dry methylene dichloride and saturated at 0° with dry hydrogen chloride. The *p*-nitrobenzoic acid precipitated is filtered off under anhydrous conditions, and the 1-chloro derivative II is obtained in quantitative yield by evaporation of the solvent and can be used for the ensuing condensation without further purification.

The invention is illustrated by the following Examples:

Example 1.

4'-deoxy-daunomycin

A solution of 1.5 g of daunomycinone in 150b ml of anhydrous methylene dichloride containing 0.75 g of 2,3,4,6-tetradeoxy-3-trifluoroacetamido-L-threo-hexopyranosyl chloride II, is vigorously stirred in the presence of 10 g of molecular sieve (4Å Merck) with 0.77 g of $AgSO_3CF_3$ in 20 ml of anhydrous diethyl ether. "E Merk" is a Trade Mark. After two hours at room temperature, the reaction mixture is neutralized with a saturated aqueous solution of sodium bicarbonate and the organic phase, separated, is evaporated under vacuum. The residue is dissolved in 50 ml of 0.1 N aqueous sodium hydroxide, and after 30 minutes at 0°C the solution is adjusted to pH 3.5 and repeatedly extracted with chloroform to eliminate the aglycone. Then the solution is adjusted to pH 8.3 and extracted with chloroform until the extracts are no longer coloured. The combined organic extracts, dried over anhydrous sodium sulphate, are concentrated to a small volume and acidified to pH 4.5 with 0.1 N methanolic hydrogen chloride. The addition of an excess of diethyl ether afforded 4'-deoxy-daunomycin as the hydrochloride: m.p. 160—4°C (decomposed) TLC on Merck Kieselgel F_{254} plate

using a chloroform-methanol-water (150:42:6 v/v) solvent system: Rf 0.47; $[\alpha]_D^{20} = +296°$ (C=0.50 MeOH).

Example 2.
4'-deoxy-adriamycin

A solution of 4'-deoxy-daunomycin, prepared as in Example 1, in a mixture of methanol and dioxan is treated with bromine to give a 14-bromo-derivative. Treatment with an aqueous solution of sodium formate at room temperature for 100 h gives 4'-deoxy-adriamycin which is isolated as the hydrochloride; m.p. 163°C with decomposition; $[\alpha]_D^{20} = +320°$ (c=0.05 MeOH); TLC on Merck Kieselgel HF buffered at pH 7 with phosphate M/15 using methylene dichloride-methanol-water (10:2:02 v/v) solvent system Rf 0.13.

Further Examples

The condensation of compound II with the following anthracyclinones: 1,4-dimethyl - 4 - demethoxydaunomycinone, 1,4 - dichloro - 4 - demethoxy - daumomycinone, 1,4 - dibromo - 4 - demethoxydaunomycinone, 2,3 - dimethyl - 4 - demethoxydaunomycinone, 2,3 - dichloro - 4 - demethoxydaunomycinone, 2,3-dibromo - 4 - demethoxydaunomycinone, 1 - methoxy - daunomycinone, gives the corresponding 4' - deoxy - glycosides.

Biological activity

The antitumour activity of the novel antibiotic compounds of the invention, 4'-deoxy-daunomycin and 4'-deoxy-adriamycin, was evaluated on several transplanted tumours in mice in comparison with the known antitumour agents daunomycin and adriamycin.

Inbred BDF_1 mice were intraperitoneally inoculated with 10^5 leukemia cells per mouse and then treated (interaperitoneally) one day after the tumour inoculation with varying doses of the compounds under examination. The average survival time percentage and the number of long term survivors are given in Table 1.

TABLE 1

Compound	Action on L_{1210} Leukemia			
	Dose mg/kg	Average Survival Time (%)	Long Term Survivors (after 60 days)	Toxic Deaths
Daunomycin	2	162	–	–
	4	162	–	–
	6	156	–	4/10
4'-Deoxy-daunomycin	4	162	–	–
	8	187	–	1/10
	16	162	–	8/10
Adriamycin	2.5	145	–	–
	5	155	2/20	–
	10	163	2/20	3/18
4'-Deoxy-Adriamycin	1	155	–	–
	2	162	–	–
	4	177	2/10	–
	5	133	1/10	6/10
	10	66	–	10/10

Transplanted Gross Leukemia

Inbred C_3H female mice were intravenously inoculated with a suspension of Leukemia lymphonodes and spleen cells (2×10^6 Leukemia cells/mouse) and treated intravenously on days 1, 2 and 3 after inoculation with the compounds under examination. The average survival time percentage is given in Table 2.

TABLE 2

Compound	Action on Transplanted Gross Leukemia			
	Dose mg/kg	Average Survival Time (%)	Long Tem Survivors (after 45 days)	Toxic Deaths
Daunomycin	3.5	128	–	–
	4.5	143	–	–
	5.5	171	–	–
4'-Deoxy-Daunomycin	2	128	–	–
	4	157	–	2/10
	8	100	–	9/10
Adriamycin	3.5	183	–	–
	4.5	200	–	–
	5.5	216	3/10	–
4'-Deoxy-Adriamycin	1.75	200	–	–
	2.25	233	1/10	–
	2.75	233	–	1/9

Ascites lymphocytic P_{388} Leukemia

Male CDF_1 mice were intraperitoneally inoculated with 10^6 leukemia cells per mouse and treated intraperitoneally on the day one after inoculation with different doses of the compound under examination. The average survival time percentages are given in Table 3.

TABLE 3

Compound	Action on P_{388} Leukemia	
	Dose mg/Kg	Average Survival Time (%)
Adriamycin	2.5	218
	5	209
	10	264
4'-Deoxy-Adriamycin	2	204
	4	218
	6	236
	8	245

Solid sarcoma 180

Swiss CD_1 mice were subcutaneously grafted with fragments of neoplastic (Solid Sarcoma 180) tissue and treated intravenously on days 1,2,3,4 and 5 after inoculation with different doses of the compound under examination. The growth of the tumour was evaluated by caliper measurement on the 11th day after the tumour implants. The results are given in Table 4.

TABLE 4

Compound	Activity on Solid Sarcoma 180		
	Dose mg/Kg	Tumour Weight (g)	T/C %
Adriamycin	1.6	0.890	52
	2.0	0.875	51
4'-Deoxy-adriamycin	0.8	0.810	47
	1.0	0.790	46

T/C% = mean tumour weight of treated mice/mean tumour weight of control mice × 100.

WHAT WE CLAIM IS:—

1. A process of preparing a glycoside of formula IV as herein defined which comprises condensing an aglycone of formula I as herein defined with a halo sugar of formula II as herein defined, and removing the protecting group under alkaline conditions.

2. A process according to claim 1 in which the condensation is effected in an organic solvent in the presence of silver trifluoromethanesulphonate and a dehydrating agent.

3. A process according to claim 1 or claim 2 as herein described in Example 1.

4. A process according to any of claims 1 to 3, the process further comprising converting the product into its adriamycin analogue by bromination and hydrolysis of the resulting 14-bromo-derivative.

5. A glycoside of formula IV as herein defined, or an adriamycin analogue thereof.

6. 4'-Deoxy-daunomycin.

7. 4'-Deoxy-adriamycin.

8. 1,4-Dimethyl-4-demethoxy-4'-deoxy-daunomycin.

9. 1,4-Dimethyl-4-demethoxy-4'-deoxy-adriamycin.

10. 1,4-Dichloro-4-demethoxy-4'-deoxy-daunomycin.

11. 1,4-Dichloro-4-demethoxy-4'-deoxy-adriamycin.

12. 1,4-Dibromo-4-demethoxy-4'-deoxy-daunomycin.

13. 1,4-Dibromo-4-demethoxy-4'-deoxy-adriamycin.

14. 2,3-Dimethyl-4-demethoxy-4'-deoxy-daunomycin.

15. 2,3-Dimethyl-4-demethoxy-4'-deoxy-adriamycin.

16. 2,3-Dichloro-4-demethoxy-4'-deoxy-daunomycin.

17. 2,3-Dichloro-4-demethoxy-4'-deoxy-adriamycin.

18. 2,3-Dibromo-4-demethoxy-4'-deoxy-daunomycin.

19. 2,3-Dibromo-4-demethoxy-4'-deoxy-adriamycin.

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9. 1,4-Dimethyl-4-demethoxy-4'-deoxy-adriamycin.

10. 1,4-Dichloro-4-demethoxy-4'-deoxy-daunomycin.

11. 1,4-Dichloro-4-demethoxy-4'-deoxy-adriamycin.

12. 1,4-Dibromo-4-demethoxy-4'-deoxy-daunomycin.

13. 1,4-Dibromo-4-demethoxy-4'-deoxy-adriamycin.

14. 2,3-Dimethyl-4-demethoxy-4'-deoxy-daunomycin.

15. 2,3-Dimethyl-4-demethoxy-4'-deoxy-adriamycin.

16. 2,3-Dichloro-4-demethoxy-4'-deoxy-daunomycin.

17. 2,3-Dichloro-4-demethoxy-4'-deoxy-adriamycin.

18. 2,3-Dibromo-4-demethoxy-4'-deoxy-daunomycin.

19. 2,3-Dibromo-4-demethoxy-4'-deoxy-adriamycin.

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