Tetrahedron Vol 47, No 25 pp 4435-4440, 1991
Printed in Great Britain

0040-4020/91 $3 00+ 00

A NEW CLASS OF ANTHRAQUINONE-ANTHRONE-*C*-GLYCOSIDES FROM *ASPHODELUS ramosus* TUBERS.

MATTEO ADINOLFI, ROSA LANZETTA,* CARMELA ELSA MARCIANO, MICHELANGELO PARRILLI
Dipartimento di Chimica Organica e Biologica, Universita' di Napoli
Via Mezzocannone 16, 80134 Napoli, Italy

ALFONSO DE GIULIO
Istituto per la Chimica delle Molecole di Interesse Biologico del CNR,
Via Toiano 6, Arco Felice, Napoli, Italy

(*Received in UK* 11 *January* 1991)

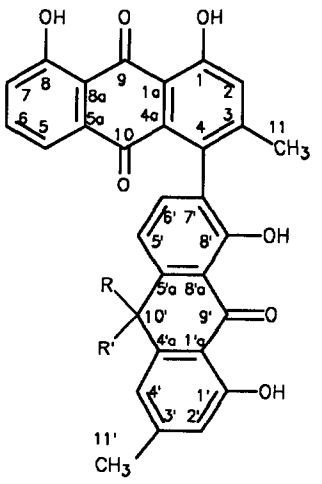
Abstract- The isolation and the structural determination of six new 7'-(chrysophanol-4-yl)-chrysophanol-10'-anthrone 10'-*C*-glycosides **1-6** from *Asphodelus ramosus* tubers are reported

Introduction

The ethereal extract of *Asphodelus ramosus* (Liliaceae) tubers was found (1,2) to be a source of a new class of *C*-glycosides characterized by a bianthrone aglycone As this extract showed an interesting LC_{50} value (4 5 ppm) to the *Artemia salina* bioassay, we submitted it to further analysis and obtained the six novel *C*-glycosides **1-6**, closely related to those previously isolated but constituting a further class of *C*-glycosides with anthraquinone-anthrone aglycone In this paper we describe their isolation and caracterization, and, for some of these compounds, the *Artemia salina* bioassay The structural elucidation was mainly based on spectral (^{1}H-, ^{13}C-NMR, UV, FAB-ms) evidence and confirmed by chemical degradation

Aglycone structure

The close similarity of ^{1}H-NMR data in Table 1 immediately suggested that all of compouds **1-6** shared the same aglycone, whose anthraquinone nature was indicated by UV absorption (λ_{max}^{MeOH} 425, 370, 286, 254 nm) and confirmed by further NMR analysis The main structural features drawn from ^{1}H-NMR data by homonuclear decoupling and NOE experiments, can be summarized as follows a) three vicinal protons, b) a pair of *meta*-protons, separated by a methyl group, c) a proton *ortho* to a second methyl group, d) four chelated phenolic hydroxyl protons, e) a proton, in the range 4 63-4 76 ppm, coupled with another one occurring in the carbinolic-proton chemical shift zone, f) two protons clearly appearing as an *ortho*-coupled system for compounds **1-3** and as a broad singlet in the case of **4-6** Actually, on the basis of chemical evidence (see below), also this latter signal was attributed to a pair of protons *ortho* located



1 R = H, R' = *C*-α-rhamnopyranosyl
2 R = H, R' = *C*-β-xylopyranosyl
3 R = H, R' = *C*-β-antiaropyranosyl
4 R = H, R' = *C*-α-arabinopyranosyl
5 R = *C*-β-xylopyranosyl, R' = H
6 R = H, R' = *C*-β-quinovopyranosyl
7 R, R' = O

The ^{13}C-NMR data (Table 2), defined on the basis of on-resonance and DEPT experiments, displayed signals indicating a) eight aromatic methine carbons, b) sixteen aromatic quaternary carbons, c) three unsaturated keto groups, d) two methyl groups, e) a methine carbon resonating in the range 44 8-46 3 ppm

The analogy of these features with the spectral NMR data of the related metabolites previously reported (1,2) suggested an aglycone structure for **1-6** with a moiety of chrysophanol linked to a moiety of chrysophanol-10-anthrone

Confirmatory chemical evidence for this suggestion was obtained from the analysis of the reaction products of the spontaneous oxidative fission of the *C*-glycoside bond performed for **1-5** In the reaction crude from each compound was isolated a product whose structure was identified, but for the stereochemistry (see below), as asphodelin **7** on the basis of ^{1}H-NMR of its acetate (3)

Sugar structure

As we already emphasized for the bianthrone *C*-glycosides (2), also the class of compounds here described is characterized by a high variety of sugars, whose structures were mainly established by spectral measurements

FAB-ms spectra showed, for all compounds **1-6**, pseudomolecular ion peaks indicating the presence of only one monosaccharide in their glycones, and peaks indicating losses of 132 *m/z* for **2**, **4** and **5**, and of 146 *m/z* for **1**, **3** and **6**, in agreement with the pentose or deoxy-hexose sugar nature, respectively

The ^{1}H- and ^{13}C-NMR data, besides confirming these results, indicated the *C*-glycoside nature of all metabolites because of the lack of anomeric acetal-proton signals For compounds **2-6**, the type of each monosaccharide, its ring size and the configuration of its anomeric centre were deduced from the analysis of the ^{1}H-NMR signal patterns and from the values of the coupling constants, that

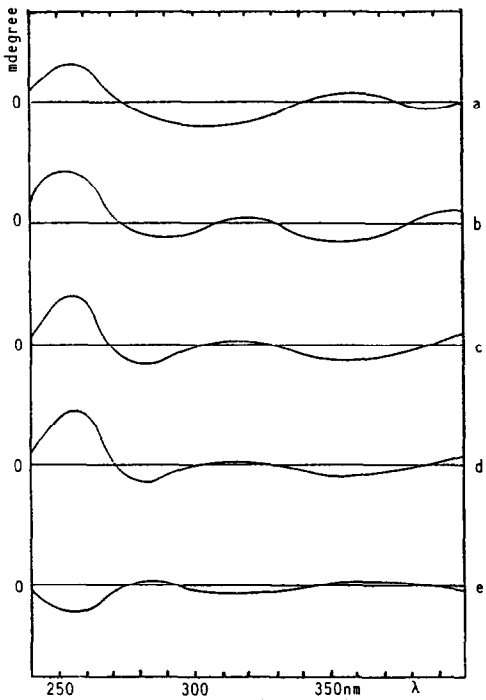


Fig 1 - CD curves of 2 (a), 5 (b), 1 (c), 4 (d), and 3 (e) in MeOH

indicated the mutual geometrical arrangement of the sugar-moiety protons Thus, for **2** and **5** the presence of β-xylopyranose was suggested, for **4** of α-arabinopyranose, for **3** of β-antiarose (β-6-deoxygulose), and for **6** of β-quinovose Experimental confirmation of these conclusions was obtained in the cases of compounds **2**, **4**, and **5** by identifying the monose (TLC, HPLC) present in the reaction crude of the spontaneous oxidative fission of the aglycone-sugar bond In addition, for both the xylose samples obtained from **2** and **5**, configuration D was inferred from the positive CD curves of their per-benzoylated derivatives(4)

In the case of **1**, the ^{1}H-NMR data measured in acetone-d_6 did not afford a safe indentification of the sugar moiety because of the overlapping of the H-1" and H-2" signals However, the oxidative fission of **1** afforded rhamnose The ^{1}H-NMR spectrum of **1** in benzene-d_6 showed well separated signals, whose $^3J_{H,H}$ analysis indicated that the sugar moiety is α-rhamnopyranose in the $^4C_1$ conformation, as was confirmed by the NOE enhancement of the H-1" signal upon irradiation of the 6"-CH_3 signal The unusual conformation assumed by the α-rhamnopyranose ring in **1** was probably due to the steric hindrance of the aglycone unit, whose equatorial orientation, differently from **2-6**, may be reached only through a shift from the usually most favoured $^1C_4$ conformation

Stereochemical aspects

The structures **1-6** have been so far defined apart from stereochemistry However, in the case of **2** and **5**, both possessing a D-β-xylopyranosyl unit, a different configuration must be assigned to their aglycone Accordingly, the CD curves of these two compouds differed in showing (Fig 1a, b) Cotton effects of opposite sign at longer wavelength and of the same sign at shorter In order to establish whether the configurational difference between **2** and **5** was due to the chirality of the 10'-centre and/or to the atropisomerism of the aglycone, the rotation of the asphodelin samples obtained by the oxidative degradation of **2** and of **5** was measured The rotation sign being positive for both samples, **2** and **5** must be epimers at C-10' In the light of this result, the analysis of CD curves might suggest that the transitions related to the longer wavelength Cotton effects were more affected by the configuration at C-10', while those related to the shorter wavelength Cotton effects were more affected by the atropisomerism of aglycone On this basis, the CD curves of **1**, **4** and **5** (Fig 1c, d, b) would indicate the same configuration at C-10' and the same aglycone atropisomer, while the CD curve of **3** (Fig 1e) would indicate the same configuration at C-10' as **2** and the aglycone atropisomer opposite

to that of **1-2** and **4-5** This latter assumption was confirmed by the negative value of the rotation of the asphodelin sample obtained by spontaneous oxidative degradation of **3**

On the basis of this result the possibility must be stressed that in the pair **2-5** one of the two compounds is an artefact of the other, as occurred for a pair of bianthrone *C*-glycosides isolated from the same source (2)

Table 1 ^{1}H-NMR data of compounds **1-7** in acetone-d_6 (apparent coupling constants in Hz)[a]

	1	1[b]	2	3	4	5	6	7
2	7 33 *bs*	7 12 *s*	7 36 *bs*	7 33 *bs*	7 34 *bs*	7 34 *bs*	7 34 *bs*	7 40 *bs*
5	7 28 *dd* (8 5, 1 2)	7 92 *d* (7 7)	7 28 *dd* (8 2, 0 9)	7 31 *d* (8 3)	7 29 *dd* (8 1, 1 3)	7 29 *dd* (7 3, 1 0)	7 29 *d* (8 2)	7 32 *dd* (8 3, 1 0)
6	7 70 *dd* (8 5, 7 6)	7 05 *t* (7 7)	7 71 *dd* (8 2, 7 3)	7 74 *dd* (8 3, 7 6)	7 72 *t* (8 1)	7 74 *t* (7 3)	7 71 *t* (8 2)	7 75 *t* (7 8)
7	7 54 *dd* (7 6, 1 2)	6 95 *d* (7 7)	7 45 *dd* (7 3, 0 9)	7 49 *d* (7 6)	7 59 *dd* (8 1, 1 3)	7 59 *dd* (7 3, 1 0)	7 45 d (8 2)	7 51 *dd* (8 3, 1 0)
2'	6 72 *d* (1 7)	6 59 *s*	6 75 *bs*	6 73 *bs*	6 72 *bs*	6 70 *bs*	6 72 *bs*	7 21 *bs*
4'	6 93 *d* (1 7)	6 87 *s*	7 08 *d* (0 9)	7 07 *bs*	6 95 *bs*	6 96 *bs*	6 96 *bs*	7 68 *d* (1 0)
5'	7 20 *d* (7 6)	7 20 *d* (7 7)	7 14 *d* (7 7)	7 15 *d* (7 6)	7 23 *bs*	7 23 *bs*	7 25 *bs*	7 57 *d* (7 8)
6'	7 26 *d* (7 6)	7 80 *d* (7 7)	7 31 *d* (7 7)	7 26 *d* (7 6)	7 23 *bs*	7 23 *bs*	7 25 *bs*	7 89 *d* (7 8)
10'	4 63 *d* (2 5)	4 64 *d* (2 2)	4 75 *d* (2 2)	4 72 *d* (3 0)	4 73 *d* (1 9)	4 69 *d* (2 2)	4 76 *bs*	-
11	2 17 *s*	2 04 *s*	2 17 *s*	2 12 *s*	2 11 *s*	2 08 *s*	2 14 *s*	2 16 *s*
11	2 40 *s*	2 06 *s*	2 40 *s*	2 40 *s*	2 38 *s*	2 39 *s*	2 41 *s*	2 52 *s*
Sugar protons								
1"	3 98	4 08 *dd* (2 2, 8 6)	3 45 *dd* (2 2, 9 5)	4 05 *dd* (8 5, 3 0)	3 35 *dd* (9 5, 2 1)[c]	3 45 *dd* (9 7, 2 2)	4 03 *d* (5 8)	
2"	3 98	4 19 *dd* (3 7, 8 6)	3 19 *t* (9 5)	3 80 *dd* (8 5, 3 0)	3 61 *t* (9 5)[c]	3 37 *t* (9 5)	3 90 *dd* (7 3, 5 8)	
3"	3 91 *t* (2 5)	3 94 *t* (3 7)	3 32 *t* (9 5)	3 92 *t* (3 0)	3 48 *dd* (9 5, 3 6)[c]	3 37 *t* (9 5)	3 22 *dd* (9 7, 7 3)	
4"	3 41 *dd* (5 3, 2 5)	3 47 *t* (3 7)	3 13 *m*	3 56 *dd* (3 5, 3 0)	3 77 m[c]	3 32 *m*	3 49 *m*	
5"a	3 33 *m*	3 64 *dq* (3 7, 7 0)	2 82 *t* (10 8)	3 60 *m*	3 91 *dd* (13 5, 2 2)[c]	2 83 *t* (10 6)	3 48 *m* (9 7)[d]	
5 b		-	3 61 *dd* (10 8, 5 7)	-	3 34 *dd* (13 5, 1 8)[c]	3 64 *dd* (10 6, 5 4)		
6	1 0 *d* (6 4)	1 07 *d* (7 0)	-	1 24 *d* (6 5)	-	-	1 24 *d* (6 4)	
Phenolic hydroxyl protons[e]								
1	12 49 *s*	12 81 *s*	12 49 *s*	12 50 *s*	12 50 *s*	12 50 *s*	12 50 *s*	12 47 *s*
8	12 24 *s*	12 65 *s*	12 17 *s*	12-19 *s*	12 18 *s*	12 13 *s*	12 20 *s*	12 25 *s*
1'	11 95 *s*	12 35 *s*	11 96 *s*	11 97 *s*	11 96 *s*	11 95 *s*	12 00 *s*	11 90 *s*
8'	11 82 *s*	12 09 *s*	11 86 *s*	11 90 *s*	11 89 *s*	11 82 *s*	11 85 *s*	11 80 *s*

[a] Assignments of **1-6** were obtained by comparison with **7** and on the basis of decoupling and NOE experiments

[b] Measured in benzene-d_6

[c] These data were measured in chloroform-d_1 where the signals appeared well resolved

[d] Measured upon irradiation of H-6 at 1 24 ppm

[e] Assignments in each column may be interchanged

Table 2 ^{13}C-NMR chemical shifts (ppm) and DEPT of compounds **1**, **2**, **4**, **5** and **7** in acetone-d_6[a]

Carbons	DEPT	1	2	4	5	7
9, 9	C	195 4, 194,1	195 4, 194 1	195 4, 193 8	195 2, 193 9	193 9
10	C	183 0	182 7	[b]	182 6	182 1[c]
1, 8, 1', 8'	C	163 9, 162 9, 162 7, 160 5	163 3, 162 9, 162 7, 159 7	163 4, 162 9, 162 8, 160 2	163 2, 162 6, 160 7, 160 5	163 5, 162 8, 160 7, 160 5
3, 3'	C	151 4, 148 8	151 5, 148 1	151 1, 148 9	151 4, 148 8	150 6, 150 4
4'a, 5'a	C	147 6, 142 3	145 8, 142 9	147 2, 141 6	147 1, 141 2	134 6[d], 133 4[d]
6	CH	138 2	138 2	138 2	138 2	138 3[e]
7'	C	135 6	136 7	135 5	135 3	135 1[d]
6'	CH	135 3	135 5	135 3	135 5	137 4[e]
4a, 5a	C	132 8, 132 3	132 6, 132 2	133 0, 130 7	132 6, 132 0	132 1 131 3
4	C	128 7	128 3	128 8	128 5	[b]
2, 5, 7	CH	125 5, 124 4, 121 9	125 6, 124 3, 122 4	125 6, 124 4, 121 4	125 5, 124 3, 121 4	125 8, 124 8, 124 6
2' 4'	CH	120 5, 120 5	120 2, 119 7	120 7 120 7	120 5, 120 3	121 6[f] 120 6[f]
5'	CH	116 5	116 5	116 5	116 6	120 3[f]
10'	CH	46 3	45 1	45 1	44 8	182 7[c]
11 11'	CH_3	22 1, 21 4	22 2, 21 3	22 1, 21 4	22 0, 21 3	22 1, 21 3
Sugar carbons						
1	CH	[g]	87 7	[h]	86 9	
2"	CH	[g]	80 2	[h]	79 8	
3'	CH	[g]	71 4	[h]	71 2	
4"	CH	[g]	71 0	[h]	70 7	
5"	CH/CH_2	[g]	71 3	[h]	70 9	
6"	CH_3	17 8				

[a] Assigments were obtained by comparison with data reported in refs 1 and 2 for bianthrone *C*-glycosides
[b] Not detected
[c,d,e,f] Interchangeable assignments
[g] Not assigned signals at 79 8, 76 0, 74 5, 74 1 and 66 6 ppm
[h] Not assigned signals at 87 2, 76 0, 71 7, 70 6 and 68 8 ppm

EXPERIMENTAL

^{1}H- (400 135) and ^{13}C-NMR (100 614) spectra were recorded in acetone-d_6 with a 400-AM FT-NMR spectrometer (Bruker), equipped with dual probe UV spectra were measured in methanol with a Cary-210 spectrometer CD curves were measured in methanol with a JASCO J-600 dichrograph Mass spectra were recorded with a VG ZAB-2SE instrument equipped with a FAB source Rotations were determined in methanol solutions on a Perkin Elmer mod 141 polarimeter Hplc was performed with a Varian 5060 instrument using a UV detector

Isolation of compounds **1-6** The ether extract (3 g) of *Asphodelus ramosus* tubers was submitted to DCCC separation as reported (1,2) Fraction A (1 8 g) was chromatographed on a silica gel (200 g) column with a Chromatospac Prep 10 apparatus (Jobin Yvon) Elution with chloroform-methanol (97 3) gave five fractions in order of increasing polarity *a* (200 mg), *b* (50 mg), *c* (150 mg), *d* (130 mg), and *e* (800 mg)

Fraction *a* was submitted to further column chromatography on silica gel Two main groups of fractions were obtained by elution with 99 1 and 98 2 chloroform-methanol, respectively Upon PLC on silica gel with 9 1 chloroform-methanol of the less polar group (25 mg), **4** was yielded as a yellow amorphous solid ^{1}H- and ^{13}C-NMR see Tables FAB-ms *m/z* 625 $(MH)^+$, 493 $(MH - 132)^+$ and 475 $(MH - 132 - 18)^+$ PLC on silica gel with 95 5 chloroform-methanol of the more polar fraction (75 mg) gave **1** (40 mg) as an amorphous solid ^{1}H- and ^{13}C-NMR see Tables FAB-ms *m/z* 639 $(MH)^+$, 493 $(MH-146)^+$

PLC on silica gel with 95 5 chloroform-methanol of fraction *b* gave **1** (14 mg) and a fraction from which **3** (2 mg) and **6** (2 mg) were obtained as amorphous solids by HPLC (Lichrosphere RP-18, 85 15 methanol-water 1 0 ml/min , UV detection at 260 nm) ^{1}H-NMR see Table 1 FAB-ms of **3** *m/z* 639 $(MH)^+$, 493 $(MH-146)^+$ FAB-ms of **6** *m/z* 639 $(MH)^+$, 493 $(MH-146)^+$

Compound **5** (25 mg), amorphous solid, was obtained from fraction *c* by subsequent HPLC (Lichrosphere RP-18, 85 15 methanol-water, 1 0 ml/min , UV detection at 260 nm) and PLC on silica gel with 95 5 chloroform-methanol ^{1}H- and ^{13}C-NMR see Tables FAB-ms *m/z* 625 $(MH)^+$, 493 $(MH - 132)^+$ and 475 $(MH - 132 - 18)^+$

PLC on silica gel with chloroform-methanol (95 5, 6 runs) of fraction *d* gave compound **2** (30 mg) as an amorphous solid ^{1}H- and ^{13}C-NMR see Tables FAB-ms *m/z* 625 $(MH)^+$, 493 $(MH - 132)^+$ and 475 $(MH - 132 - 18)^+$

Spontaneous alteration of compounds **1-5** Samples of **1-5** were separately left in acetone in an NMR tube for a long period (one-two months) TLC (silica gel, 95 5 chloroform-methanol) of each mixture revealed the presence of other products PLC on silica gel of each crude from compounds **1**, **2**, **4** and **5** afforded dextrorotatory asphodelin **7** and rhamnose, xylose, arabinose, and xylose, respectively In the case of compound **3** only asphodelin (levorotatory) was isolated

Biological tests Brine shrimp (*Artemia salina*) assays were performed in DMSO (1% final volume) using 10x3 animals/dose suspended in artificial sea water (5 ml) as reported in ref 5 After 24 h the data obtained were analyzed by the Finney program (6) which yields LC_{50} values with 95% confidence intervals LC_{50} values of 4 2, 50 and 2 ppm for the ether extract from the tubers and pure **1** and **2**, respectively, were found

ACKNOWLEDGEMENTS

NMR spectra were taken at the Centro Interdipartimentale di Metodologie Chimico-Fisiche, Universita' di Napoli We thank dr A Scopa (Universita' della Basilicata, Potenza) for running the CD curves, the Servizio di Spettrometria di Massa CNR-Universita' di Napoli for the mass spectral data and Ministero Pubblica Istruzione, Roma, for financial support of this work

REFERENCES

1 M Adinolfi, M M Corsaro, R Lanzetta, M Parrilli, A Scopa, *Phytochemistry*, **1989** *28* 284

2 R Lanzetta, M Parrilli, M Adinolfi, T Aquila, M M Corsaro, *Tetrahedron*, **1990** *46* 1287

3 A Yagi, K Makino, I Nishioka, *Chem Pharm Bull* , **1978** *26* 1111

4 N Harada, K Nakanishi, *Circular Dichroic Spectroscopy - Exciton Coupling in Organic Stereochemistry* - Oxford University Press, 1983

5 B N Meyer, N R Ferrigni, J E Putnan, L B Jacobsen, D E Nicols, L McLaughlin, *Planta Med* , **1982** *45* 31

6 D J Finney, *Probit Analysis*, 3rd ed , Cambridge University Press, Cambridge, **1971**