

18,20-Oxido-20,22-dihydroneeriifolin, an Unusual Oxygenated Cardanolide¹

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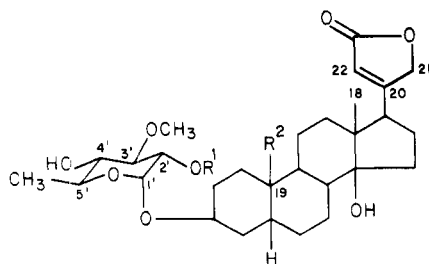
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Three new monoglycosidic substances were obtained from the enzymatic hydrolysis products of the mixture of triglycosides isolated from *Thevetia thevetoides* Schum. It was established, through degradative experiments and spectroscopic techniques, that the more abundant compounds **2a** and the 2'-acetate thereof **2b** were members of a heretofore unknown class of 18-oxygenated cardanolides, both of which were isolated as mixtures of 20(*R*) and 20(*S*) isomers. The least abundant compound was shown to be peruvoside 2'-acetate (**1d**) by transformation into ruvoside (**1e**).

It was recently reported² that the enzymatic hydrolysis of the mixture of cardanolide triglycosides isolated from the seeds of *Thevetia thevetoides* Schum., growing in Mexico, gave two monoglycosides of unknown structure, in addition to neriifolin (**1a**) and neriifolin monoacetate



- 1a, R¹ = H; R² = CH₃
 b, R¹ = CH₃CO; R² = CH₃
 c, R¹ = H; R² = CHO
 d, R¹ = CH₃CO; R² = CHO
 e, R¹ = H; R² = CH₂OH

(**1b**). This paper describes the details of the isolation and structure determination of the above compounds and of a third, previously unreported, very minor monoglycoside.

Column chromatographic separation, on silica gel, of the monoglycosidic mixture obtained in the manner described previously² resulted in the successive elution of five principal components: neriifolin monoacetate (**1b**) (20%), unknown A (20%), unknown B (2.3%), neriifolin (**1a**) (31%), and unknown C (9%).

The microanalytical and mass spectral data showed that the least abundant compound (**B**) had a molecular formula of C₃₂H₄₀O₁₀, and the UV, IR, ¹H NMR (see Experimental Section), and ¹³C NMR (Table II) spectra established the presence of butenolide, acetate, and aldehyde moieties therein. These data, and the knowledge that peruvoside (**1c**) is reported to occur in various *Thevetia* species,³ suggested that substance **B** was probably peruvoside 2'-acetate (**1d**). This conclusion was supported by the transformation of compound **1d** into ruvoside (**1e**), and the monoacetate thereof, on reduction with sodium borohydride in methanol solution.

The mass spectral and microanalytical data established that compounds **A** and **C** possessed the molecular formulas C₃₂H₄₈O₁₀ and C₃₀H₄₆O₉, respectively, and that the former was probably a mono-*O*-acetyl derivative of the latter. The close structural kinship of compounds **A** and **C** was confirmed by acetylation of these substances to the same diacetate, and therefore degradative studies were confined to compound **C**. The solution (CHCl₃) IR spectrum of this compound had a single strong absorption at 1791 cm⁻¹ with a low intensity shoulder at 1758 cm⁻¹. This was different from the three-band pattern typically observed⁴ for cardanolides in the 1600–1800-cm⁻¹ region (e.g., digitoxigenin: 1789, 1747, 1619 cm⁻¹) and was consistent with the presence of a saturated γ -lactone system. The weak intensity of the UV spectrum [218 nm (ϵ 700)]⁵ and the absence of olefinic absorptions in the NMR spectrum (Table I) confirmed that a butenolide moiety was not present in compound **C**. In addition, even though the melting ranges of substances **A**, **C**, and the derived diacetate were narrow, the NMR spectra thereof were very complex and more consistent with a two-component mixture (ca. 1:3). Given the presence of a saturated γ -lactone moiety and NMR evidence for only one angular methyl group, carbon-18 thus became a logical site for one of the points of attachment of the extra oxygen atom in compound **C**, while carbons 20, 21, or 22 were the most reasonable locations for the remaining carbon-oxygen bond. Substance **C** was then subjected to the previously reported² degradation sequence in order to remove the thevetose residue. The ¹H and ¹³C (Table II) NMR spectra of the aglycone (see below) thus obtained clearly established that compound **C**, and therefore compound **A**, possessed the spiro structures **2a** and **2b**, respectively, and that these substances consisted of a mixture of two epimers at C-20. Thus chromic acid oxidation of the spiro triol **2a**, in a two-phase system, gave a 1:4 mixture of the

(4) Jones, R. N.; Gallagher, B. S. *J. Am. Chem. Soc.* **1959**, *81*, 5242. Jones, R. N.; Angell, C. L.; Ito, T.; Smith, R. J. D. *Can. J. Chem.* **1959**, *37*, 2007.

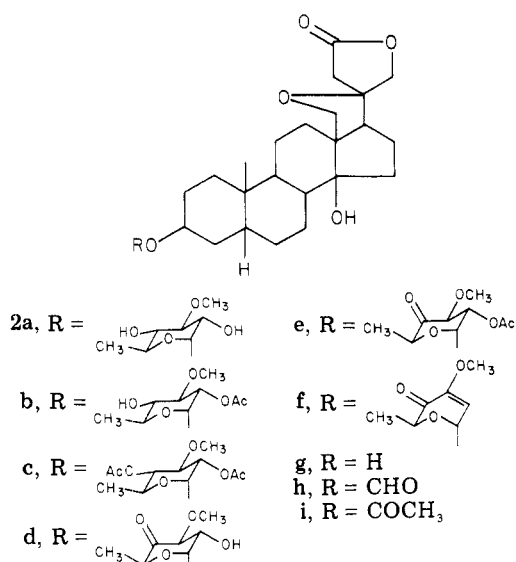
(5) The origin of the UV absorption observed for **A**, **C**, and the diacetate thereof is unknown. Butyrolactone was shown to possess a weak band at 214 nm (ϵ 26) similar to that reported⁶ for esters, and therefore these chromophores can be ruled out as important contributors to the observed extinction coefficient. One rationalization of this absorption is the presence of a small (ca. 5%) equilibrium amount of the hydroxybutenolide (**3a**), the concentration of which would be more significant in polar solvents. No olefinic absorptions were found, however, when the ¹³C NMR spectrum of **A** (0.150 g sample, 15 800 scans) was measured in Me₂SO-*d*₆. A concentration of 5% of the open chain form would easily have been detectable under these conditions.

(6) Calvert, J. G.; Pitts, J. N. "Photochemistry", Wiley: New York, 1966; pp 429 and 434–441, and references therein.

(1) Contribution No. 530 from the Syntex Institute of Organic Chemistry and No. 100 from the Analytical Research Department.

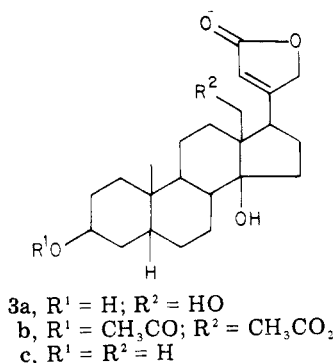
(2) Cruz, A.; Garcia, I.; Iriarte, J.; Muchowski, J. M.; Regla, I. *J. Org. Chem.* **1977**, *42*, 3580.

(3) (a) Rangaswami, S.; Rao, E. V. *J. Sci. Ind. Res., Sect. B* **1959**, *18*, 443. (b) Rangaswami, S.; Rao, E. V. Indian Patent, **1960**, *66*, 196. *Chem. Abstr.* **1961**, *55*, 14832. (c) Rangaswami, S.; Rao, E. V. *Proc. India Acad. Sci., Sect. A* **1961**, *54*, 345. (d) Lang, H.; Sun, N. *Yao Hsueh Hsueh Pao* **1964**, *11*, 464. *Chem. Abstr.* **1966**, *62*, 9465.



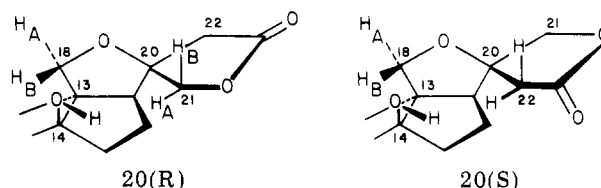
formate **2h** and the ketol **2d**. Acetylation of the β -ketol **2d** yielded the acetate **2e** which was partially transformed into the enone **2f** under the reaction conditions. Mild acidic hydrolysis of this enone provided the mixture of aglycones **2g** referred to above.

Inasmuch as substance **2g** is the Michael-type adduct derived from the hypothetical 18-hydroxydigitoxigenin (**3a**), it should be possible to effect a reversal of the ad-



dition under suitable conditions. Indeed, acetylation of the acetate **2i** under forcing conditions (acetic anhydride/triethylamine/4-(dimethylamino)pyridine/reflux) gave 18-acetoxyl-digitoxigenin 3-acetate (**3b**). Furthermore, DBN (1,5-diazabicyclo[4.3.0]non-5-ene) catalyzed transesterification of the diacetate **3b**, in methanol solution, regenerated the monoacetate **2i**, but in this case fractional crystallization of the product gave a material which consisted of more than 90% of that epimer which had been the less abundant component of the mixture derived from the glycoside **2a** (i.e., prior to cleavage of the spiro system).⁷ The availability of this epimer of the acetate **2i** in an essentially pure form greatly facilitated the interpretation of the proton and carbon NMR spectra of many of the epimeric mixtures of compounds described herein and hence the deduction of the structures thereof. The ¹³C spectra of the epimers of **2i** not only showed the expected downfield shift for carbon 18 and shielding of carbons 20 and 22, as compared to digitoxigenin acetate (**3c**), but in addition it permitted an unambiguous assignment of the

stereochemistry of each epimer. Thus, for the less abundant component of the mixture of degradation products **2i**, the resonance for C-21 is located upfield by 1.6 ppm, and that for C-22 is deshielded by 5.5 ppm in comparison to the corresponding absorptions for the major epimer. Therefore, the disposition of C-21 and C-16 must be cis in the minor epimer and trans in the major epimer⁸ which corresponds to the *R* and *S* configurations, respectively, at C-20 (see partial formulas below). The 20(*R*) and 20(*S*) isomers of compound **2i** were also readily



identifiable by the distinctly different NMR absorptions of the protons on C-21 and C-22. For the 20(*S*) (major) epimer, the hydrogens on C-21 resonated as a singlet (accidental equivalence) at δ 4.38 while those situated at C-22 appeared as an AB quartet ($J_{AB} = 17.7$ Hz) with A at δ 2.41 and B at δ 2.73. In contrast, for the 20(*R*) (minor) isomer, the absorption of the C-22 hydrogens occurred as a singlet at δ 2.61 and the protons on C-21 appeared as an AB quartet ($J_{AB} = 9.7$ Hz) with A at δ 3.42 and B at δ 4.31. The high field half of this quartet is tentatively assigned to the 21-pro *R* hydrogen H_A⁹ which apparently is ideally juxtaposed to be strongly shifted upfield (with respect to the 21-pro *S* hydrogen) by the anisotropic effect¹⁰ of the C-16,17 single bond. The relatively small chemical shift difference (δ 0.33) between the protons on C-22, for the 20(*S*) epimer, must be a reflection of a geometry in which the interaction between the 22-pro *R* hydrogen and the 16,17-carbon-carbon bond is reduced.

While the occurrence of 19-oxygenated cardenolides and bufadienolides is relatively widespread,¹¹ oxygenation of these systems at C-18 is exceedingly rare. Indeed, to our knowledge, no cardiac glycosides of this type have heretofore been described.¹²

Experimental Section

The melting points were determined in a Mel-Temp melting point apparatus and are not corrected. The infrared spectra were measured with a Perkin-Elmer Model 237 grating infrared spectrophotometer. The ultraviolet spectra were recorded on a Perkin-Elmer Model 402 ultraviolet visible spectrophotometer. The ¹H NMR spectra were measured with a Varian HA-100 spectrometer and are expressed as parts per million (δ) from internal tetramethylsilane. The ¹³C NMR spectra were recorded in chloroform solution with a Bruker WH-90 Pulse Fourier Transform spectrometer. The mass spectra were measured with an Atlas CH-4 spectrometer.

Isolation of Peruvoside 2'-Acetate (1d), 18,20-Oxido-20,22-dihydroneeriifolin (2a), and 18,20-Oxido-20,22-dihydroneeriifolin 2'-Acetate (2b) from *Thevetia thevetoides*

(8) Verheyden, J. P. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1975**, *97*, 4386. Arróniz, C. E.; Gallina, J.; Martínez, E.; Muchowski, J. M.; Rooks, W. H.; Velarde, E. *Prostaglandins* **1978**, *16*, 47; and references therein.

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(10) Zürcher, R. F. in "Progress in Nuclear Magnetic Resonance Spectroscopy"; Emsley, J. W.; Feeney, J.; Sutcliffe, L. H., Eds.; Pergamon Press: Elmsford, N.Y., 1967; Vol. 2, pp 205-257.

(11) Tamm, C. in "Progress in the Chemistry of Natural Products"; Zechmeister, I., Ed.; Springer-Verlag, Vienna, 1957; Vol. 14, pp 72-139. Reichstein, T. *Naturwissenschaften* **1967**, *54*, 53.

(12) Neither the spiro compounds **2a-i** nor 18-acetoxyl-digitoxigenin acetate (**3b**) had appreciable cardiotonic activity in vitro or in vivo.

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(7) The crude product, before fractional crystallization, consisted of ca. one part of the 20(*S*) and two parts of the 20(*R*) epimers. When the 90+% pure 20(*R*) epimer was subjected to the transesterification conditions, a 65:35 20(*R*):20(*S*) mixture was obtained. A 1:1 mixture also gave the 65:35 *R*:*S* isomer ratio and must, therefore, correspond to the equilibrium composition of the isomers.

Table I. NMR Data^a for 18,20-Oxido-20,22-dihydroneeriifolin and Related Compounds

compd no.	1'-H	2'-H	3'-H	3'-OCH ₃	4'-H	5'-H	5'-CH ₃	3 α -H	20(S)	
									H-18A	H-18B
2a ^b	4.63 d, <i>J</i> = 3 Hz			3.48	2.88 t, <i>J</i> \approx 8.5 Hz		1.07 d, <i>J</i> = 6 Hz	3.97 m		
2b	5.04 d, <i>J</i> = 3.8 Hz	4.62 q, <i>J</i> = 3.8, 9.9 Hz		3.56	3.16 t, <i>J</i> = 9 Hz		1.24 d, ^c <i>J</i> = 6 Hz	3.85 m	3.38 d, <i>J</i> = 10 Hz	4.16 d, <i>J</i> = 10 Hz
2c	5.07 d, <i>J</i> = 3.7 Hz		3.62 t, <i>J</i> = 9.6 Hz	3.42	4.72 t, <i>J</i> = 9.6 Hz		1.10 d, ^d <i>J</i> = 6 Hz	3.85 m		4.17 d, <i>J</i> = 9.8 Hz
2d	5.04 d, <i>J</i> = 3.6 Hz	3.81 q, <i>J</i> = 3.6, 10 Hz		3.58		4.29 q, <i>J</i> = 6 Hz	1.23 d, ^e <i>J</i> = 6 Hz		3.40 d, <i>J</i> = 9.4 Hz	4.17 d, <i>J</i> = 9.4 Hz
2e	5.25 d, <i>J</i> = 4 Hz	4.91 q, <i>J</i> = 4, 10.4 Hz		3.54		4.25 q, <i>J</i> = 6 Hz	1.26 d, <i>J</i> = 6 Hz	3.94 m	3.38 d, <i>J</i> = 9.5 Hz	4.17 d, <i>J</i> = 9.5 Hz
2f	5.41 d, <i>J</i> = 4 Hz	5.68 d, <i>J</i> = 4 Hz		3.62		4.61 q, <i>J</i> = 6.6 Hz	1.36 d, <i>J</i> = 6.6 Hz	4.01 m	3.39 d, <i>J</i> = 10 Hz	4.17 d, <i>J</i> = 10 Hz
2g								4.09 m		
2h								5.22 m ^f	3.38 d, <i>J</i> = 10 Hz	4.17 d, <i>J</i> = 10 Hz
2i								5.06 m ^g	3.38 d, <i>J</i> = 9.9 Hz	4.17 d, <i>J</i> = 9.9 Hz

compd no.	20(R)		20(S)		20(R)		20(S)		20(R)		
	H-18A	H-18B	19-CH ₃	H-21A	H-21B	H-21A	H-21B	H-22A	H-22B	H-22A	H-22B
2a			0.82								
2b	3.42 d, <i>J</i> = 9.5 Hz	4.18 d, <i>J</i> = 9.5 Hz	0.90	4.34	4.34	3.91 d, <i>J</i> = 9.8 Hz	4.30 d, <i>J</i> = 9.8 Hz	2.40 d, <i>J</i> = 17.4 Hz	2.72 d, <i>J</i> = 17.4 Hz	2.60	2.60
2c		4.18 d, <i>J</i> = 9.6 Hz	0.90	4.35	4.35	3.98 d, <i>J</i> = 9.9 Hz	4.30 d, <i>J</i> = 9.9 Hz	2.41 d, <i>J</i> = 17.7 Hz	2.72 d, <i>J</i> = 17.7 Hz	2.60	2.60
2d	3.44 d, <i>J</i> = 10 Hz	4.18 d, <i>J</i> = 10 Hz	0.91	4.35	4.35	3.97 d, <i>J</i> = 10 Hz	4.32 d, <i>J</i> = 10 Hz	2.41 d, <i>J</i> = 17.3 Hz	2.72 d, <i>J</i> = 17.3 Hz	2.60	2.60
2e	3.42 d, <i>J</i> = 10.3 Hz	4.16 d, <i>J</i> = 10.3 Hz	0.91	4.35	4.35	3.97 d, <i>J</i> = 10 Hz	4.30 d, <i>J</i> = 10 Hz	2.42 d, <i>J</i> = 17.5 Hz	2.72 d, <i>J</i> = 17.5 Hz	2.60	2.60
2f	3.43 d, <i>J</i> = 10 Hz	4.19 d, <i>J</i> = 10 Hz	0.89	4.36	4.36	3.97 d, <i>J</i> = 10 Hz	4.30 d, <i>J</i> = 10 Hz	2.41 d, <i>J</i> = 17.7 Hz	2.71 d, <i>J</i> = 17.7 Hz	2.61	2.61
2g	3.43 d, <i>J</i> = 9.7 Hz	4.17 d, <i>J</i> = 9.7 Hz	0.89			3.98 d, <i>J</i> = 10.1 Hz	4.31 d, <i>J</i> = 10.1 Hz			2.62	2.62
2h	3.42 d, <i>J</i> = 9.9 Hz	4.18 d, <i>J</i> = 9.9 Hz	0.90	4.35	4.35	3.97 d, <i>J</i> = 9.5 Hz	4.31 d, <i>J</i> = 9.5 Hz	2.41 d, <i>J</i> = 17.4 Hz	2.72 d, <i>J</i> = 17.4 Hz	2.60	2.60
2i	3.42 d, <i>J</i> = 9.6 Hz	4.18 d, <i>J</i> = 9.6 Hz	0.91	4.34	4.34	3.97 d, <i>J</i> = 9.3 Hz	4.31 d, <i>J</i> = 9.3 Hz	2.41 d, <i>J</i> = 17.7 Hz	2.72 d, <i>J</i> = 17.7 Hz	2.61	2.61

^a Unless indicated otherwise, the spectra were recorded in CDCl₃. ^b Measured in Me₂SO-*d*₆. ^c Acetate methyl at δ 2.04. ^d Acetate methyls at δ 2.03, 2.07. ^e Acetate methyl at δ 2.05. ^f Formyl H at δ 8.05. ^g Acetate methyl at δ 2.02.

Schum. The defatted (hexane), powdered meal (11 kg, including the meat and the shells) was incubated in water as described previously (see ref 14, p 1479). The methanol extract (160 g) was subjected to column chromatography on silica gel (2.5 kg), and the following components were isolated in succession.

1. Neriifolin 2'-monoacetate (**1b**, 32.1 g, 20%) eluted with ethyl acetate-hexane (9:11).

2. 18,20-Oxido-20,22-dihydroneeriifolin 2'-monoacetate (**2b**, 32.0 g, 20%) eluted with ethyl acetate-hexane (11:9). This amorphous material had $[\alpha]_D -72^\circ$ (c 0.23, CHCl₃). After crystallization from

methanol-ether a solid, mp 238-240 °C, was obtained: $[\alpha]_D -80^\circ$ (c 0.30, CHCl₃); UV (MeOH) 215 nm (ϵ 870); IR (CHCl₃) 3605, 3520, 1790, 1748 cm⁻¹; mass spectrum *m/e* 592 (M⁺).

Anal. Calcd for C₃₂H₄₈O₁₀: C, 64.84; H, 8.16. Found: C, 64.79; H, 8.29.

3. Peruovoside 2'-monoacetate (**1d**, 3.6 g, 2.3%) eluted with ethyl acetate-hexane (3:2). This material crystallized as prisms, mp 223-228 °C, from ethyl acetate: $[\alpha]_D -109^\circ$ (c 0.32, CHCl₃); UV (MeOH) 223 nm (ϵ 11 800); IR (KBr) 3495, 2720, 2660, 1788, 1750 (br), 1636 cm⁻¹; NMR (CDCl₃) δ 0.94 (s, 3 H, 18-CH₃), 1.24 (d, 3 H, *J* = 6, 5'-CH₃), 2.06 (s, 3 H, CH₃CO₂), 3.17 (t, 1 H, *J* = 9 Hz, 4'-H), 3.55 (s, 3 H, OCH₃), 3.89 (m, 1 H, H-3), 4.63 (s, 1 H, *J*_{1,2'} = 3.7 Hz, *J*_{2,3'} = 9.8 Hz, 2'-H), 4.72 (q, 1 H, *J*_{21,22} = 1.4 Hz,

Table II. ^{13}C Chemical Shifts of Peruvoside 2'-Acetate 18,20-Oxido-20,22-dihydrodigitoxigenin Acetate, and Related Compounds

carbon No.	2i				
	1d	3c ^e	20(S)	20(R)	3b
1	20.94	30.49	30.49	30.53	30.49
2	28.06	25.10	25.16	25.36	25.10
3	70.34	70.42	70.38	70.38	70.38
4	28.71	30.49	30.49	30.53	30.49
5	29.45	35.73 ^a	36.83 ^a	36.80 ^a	36.80 ^a
6	25.26	26.40	26.37	26.33	26.27
7	21.24 ^a	21.20 ^b	21.65 ^b	21.62 ^b	20.84 ^c
8	42.20	41.84	43.76	43.72	42.16
9	34.88	36.87 ^a	35.79 ^a	35.79 ^a	34.30 ^a
10	50.89	35.21	37.22	37.22	35.24 ^a
11	21.84 ^a	21.29 ^b	23.70 ^b	23.67 ^b	20.94 ^c
12	39.92	40.02	37.06	37.00	35.50 ^a
13	49.74	49.67	59.30	59.17	52.63
14	85.11	85.56	84.10	84.07	85.79
15	32.28	33.22	35.14	35.11	33.35
16	26.89	26.95	25.16	25.13	28.41
17	50.75	50.97	57.61	55.49	48.70
18	15.70	15.80	71.94	71.75	62.71
19	206.14	23.73	23.86	23.86	23.73
20	174.77	174.77	87.06	88.65	174.74
21	73.60	73.54	74.35	75.94	73.83
22	117.85	117.78	40.86	35.40	117.94
23	174.77	174.77	175.00	175.13	174.74
1'	93.85				
2'	74.35				
3'	81.01				
4'	75.32				
5'	67.39				
CH ₃ CO ₂	20.94 ^d	21.52	21.49	21.49	21.29, 21.49
CH ₃ CO ₂	170.54	170.84	170.58	170.77	170.90

^{a-c} Assignments could be interchanged. ^d O-CH₃ at 60.73, 5'-CH₃ at 17.59. ^e The peak assignments for digitoxigenin acetate agree with those given by Tori et al.¹³

$J_{AB} = 18$ Hz, H-21A), 4.97 (q, 1 H, $J_{21,22} = 1.4$ Hz, $J_{AB} = 18$ Hz, H-21B), 5.05 (d, 1 H, $J_{1,2} = 3.7$ Hz, 1'-H), 5.84 (s, 1 H, $W_H = 4$ Hz, H-22), 10.02 (s, 1 H, CHO); mass spectrum m/e 572 ($M^+ - H_2O$).

Anal. Calcd for C₃₂H₄₆O₁₀: C, 65.06; H, 7.85. Found: C, 64.68; H, 7.68.

4. Neriifolin (1a, 50 g, 31.2%) eluted with ethyl acetate-hexane (13:7).

5. 18,20-Oxido-20,22-dihydroneeriifolin (2a, 15 g, 9%) eluted with ethyl acetate-hexane (1:1). The crude amorphous material ($[\alpha]_D -61.5^\circ$ (c 0.46, MeOH); UV (MeOH) 218 nm (ϵ 700)), after crystallization from methanol-water, gave a solid: mp 188-191 °C; $[\alpha]_D -56^\circ$ (c 0.375, MeOH); UV (MeOH) 219 nm (ϵ 780); IR (CHCl₃) 3585, 1791, 1758 cm⁻¹ (sh,w); mass spectrum m/e 550 (M^+).

Anal. Calcd for C₃₀H₄₆O₉: C, 65.43; H, 8.42. Found: C, 65.17; H, 8.47.

Conversion of Peruvoside 2'-Acetate (1d) into Ruvoside (1e). A solution of sodium borohydride (0.062 g) in a few milliliters of methanol was added, with stirring over a 0.5-h period, to a solution of 1d (0.150 g) in aqueous methanol (15 mL; 4:1). After the solution was left standing for 15 h at room temperature, it was made acidic with 2 N sulfuric acid, water was added, and the methanol was then removed in vacuo. The products were extracted into ethyl acetate, and the extract was washed successively with dilute sodium bicarbonate solution and water and then dried over sodium sulfate. The solvent was removed in vacuo, and the residue (0.098 g) was subjected to chromatography on neutral alumina (Fluka, Act III; 15 g). Elution with chloroform-methanol (98:2) removed a less polar (0.050 g, 51%) and a more polar product (0.040 g, 41%) from the column. The less polar material, presumably ruvoside 2'-monoacetate, was converted into the more polar product [ruvoside (1e)] on DBN-catalyzed transesterification with methanol. Crystallization of the more polar material from methanol-ether gave ruvoside: mp 230-232 °C; $[\alpha]_D -51^\circ$ (c 0.19, MeOH); UV (MeOH) 222 nm (ϵ 11 000); IR (CHCl₃) 3580, 3450,

1792 (w), 1752, 1632 (sh,w), 1624 (w) cm⁻¹ [lit.³ mp 228-230 °C; $[\alpha]_D -57.8^\circ$].

18,20-Oxido-20,22-dihydroneeriifolin 2',4'-Diacetate (2c). A solution of the monoacetate 1b (0.500 g) in pyridine (3 mL) containing acetic anhydride (1.5 mL) was left at room temperature for 48 h. Ice water was added, the product was extracted into chloroform, and the extract was washed successively with 5% hydrochloric acid solution, dilute sodium bicarbonate solution, and water. The extract was dried over sodium sulfate and evaporated in vacuo to give a solid residue (0.350 g, 80%) of the diacetate 2c. Crystallization of this substance from acetone-ether and then acetone-pentane gave the analytical sample: mp 132-134 °C; $[\alpha]_D -87^\circ$ (c 0.585, MeOH); UV (MeOH) 218 nm (ϵ 600); IR (KBr) 3585, 3540, 1795, 1755, 1640 (w) cm⁻¹.

Anal. Calcd for C₃₄H₅₀O₁₁·0.5H₂O: C, 63.47; H, 7.99. Found: C, 63.69; H, 7.91.

Acetylation of 18,20-oxido-20,22-dihydroneeriifolin (2a) under similar conditions gave 2c identical in all respects with that prepared from 2b.

Oxidation of 18,20-Oxido-20,22-dihydroneeriifolin (2a). A solution of 2a (3.49 g) in dichloromethane (175 mL) was stirred vigorously, at room temperature, with a chromic acid solution prepared from sodium dichromate dihydrate (10.5 g) in water (70 mL) containing concentrated sulfuric acid (7 mL) for 70 h. The reaction was followed by TLC on silica gel using hexane-ethyl acetate (7:3) as the developing solvent. The organic phase was separated and combined with a dichloromethane extract of the aqueous phase. The extract was washed with water, dried over sodium sulfate, and evaporated in vacuo. The residue (2.6 g) was subjected to column chromatography on silica gel (130 g) using hexane-ethyl acetate solutions of gradually increasing ethyl acetate content (from 10-20%) to develop the column. Hexane-ethyl acetate (4:1) successively eluted 18,20-oxido-20,22-dihydrodigitoxigenin 3-formate (2h, 0.194 g, 7%) and 18,20-oxido-20,22-dihydroneeriifolin-4'-one (2d).

The formate 2h was purified by crystallization from dichloromethane-ether: mp 200-201 °C; $[\alpha]_D +26.5^\circ$ (c 0.27, CHCl₃); UV 219 nm (ϵ 148); IR (KBr) 3575, 3555, 3435, 1795, 1723, 1704, 1635 (w) cm⁻¹; mass spectrum m/e 418 (M^+).

Anal. Calcd for C₂₄H₃₄O₆: C, 68.87; H, 8.19. Found: C, 68.78; H, 8.20.

The β -ketol 2d was further purified by TLC on silica gel using hexane-ethyl acetate as the developing solvent. The material thus obtained (0.80 g, 31%) was amorphous: $[\alpha]_D -67^\circ$ (c 0.114, CHCl₃); UV 214.5 nm (ϵ 910); IR (KBr) 3505, 1785, 1642, 1635 (w).

Anal. Calcd for C₃₀H₄₄O₉: C, 65.67; H, 8.08. Found: C, 65.82; H, 8.02.

Acetylation of 18,20-Oxido-20,22-dihydroneeriifolin-4'-one. A solution of the β -ketol 2d (0.338 g) in anhydrous pyridine (2 mL) containing acetic anhydride (1 mL) was left at room temperature for 18 h after which time it was poured into ice water. The mixture was extracted with ethyl acetate, and the extract was successively washed with 5% hydrochloric acid solution and dilute sodium bicarbonate solution. The extract was dried and evaporated in vacuo, and the residue (0.32 g) was resolved on TLC using hexane-ethyl acetate (1:4) as the developing solvent. The less polar component was the acetate 2e (0.097 g, 28.5%) which was amorphous: $[\alpha]_D -59^\circ$ (c 0.22, CHCl₃); IR (KBr) 3480, 1790, 1750, 1638 (w) cm⁻¹.

Anal. Calcd for C₃₂H₄₆O₁₀: C, 65.06; H, 7.85. Found: C, 65.20; H, 8.05.

The more polar, crystalline component (0.112 g, 37%) of the mixture was the enone 2f. After crystallization from dichloromethane-ether it had: mp 210-212 °C; $[\alpha]_D +22^\circ$ (c 0.17, CHCl₃); UV (MeOH) 260 nm (ϵ 4470); IR (KBr) 3600, 3420 (br) 1786, 1751 (w), 1701, 1644 cm⁻¹; mass spectrum m/e 530 (M^+).

Anal. Calcd for C₃₀H₄₂O₈·0.25H₂O: C, 67.33; H, 8.03. Found: C, 67.28; H, 7.91.

Hydrolysis of Enone 2f to 18,20-Oxido-20,22-dihydrodigitoxigenin (2g). A solution of the enone 2f (0.940 g) in methanol (10 mL) containing 0.1 N sulfuric acid (5 mL) was heated at reflux temperature for 0.5 h. The solution was made neutral by the addition of solid sodium bicarbonate, most of the solvent was removed in vacuo, and saturated sodium chloride solution was added to the residue. The product was extracted into ethyl acetate, and the extract was washed with water, dried, and

evaporated in vacuo. The residue (0.7 g) was purified by TLC on silica gel using hexane-ethyl acetate (2:3) as the developing solvent. After crystallization from dichloromethane-ether compound **2g** had: mp 231–233 °C dec; $[\alpha]_D^{25} +58^\circ$ (c 0.30, CHCl₃); UV (MeOH) 220 nm (ϵ 316); IR (KBr) 3560, 3445, (br), 1778 cm⁻¹; mass spectrum m/e 390 (M⁺).

Anal. Calcd for C₂₃H₃₄O₅·0.25H₂O: C, 69.93; H, 8.75. Found: C, 69.97; H, 8.61.

18,20-Oxido-20,22-dihydrodigitoxigenin Acetate (2i) by Acetylation of **2g**. A solution of **2g** (0.20 g) in pyridine (5 mL) and acetic anhydride (2 mL) was left at room temperature for 20 h. Water was added to the solution, and the solid was collected by filtration and purified by TLC on silica gel [hexane-ethyl acetate (3:7)]. Crystallization of the material thus obtained from ethyl acetate gave an analytical specimen: mp 234–236 °C dec (softens at 223 °C); $[\alpha]_D^{25} +19^\circ$ (c 0.31, CHCl₃); UV (MeOH) 219 nm (ϵ 170); IR (CHCl₃) 3535, 1792, 1732, 1625 (br, w); mass spectrum m/e 432 (M⁺).

Anal. Calcd for C₂₅H₃₆O₆: C, 69.42; H, 8.39. Found: C, 69.48; H, 8.47.

18-Acetoxydigitoxigenin 3-Acetate (3b). A solution of the acetate **2i** (0.490 g) in triethylamine (50 mL) containing acetic anhydride (10 mL) and 4-dimethylaminopyridine (0.1 g) was heated at reflux temperature for 18 h. The solution was poured into ice water and extracted with ethyl acetate. The extract was washed with water to neutrality, dried, and evaporated in vacuo. The residue was purified by TLC on silica gel [ethyl acetate-hexane (3:2)], and the material thus obtained (0.27 g) was subjected to another purification by TLC. The amorphous powder (0.145 g, 27%) was pure: $[\alpha]_D^{25} +48^\circ$ (c 0.224, CHCl₃); UV (MeOH) 219 nm (ϵ 14 100); IR (KBr) 3495, 1785 (m), 1743, 1629 (w) cm⁻¹; NMR (CDCl₃) δ 0.96 (s, 3 H, 19-CH₃), 2.01 (s, 3 H, CH₃CO₂), 2.04 (s, 3 H, CH₃CO₂), 3.98 (d, 1 H, J_{AB} = 11.7 Hz, H-18 α), 4.44 (d,

1 H, J_{AB} = 1.7 Hz, H-18B), 4.89 (t, 2 H, J_{21,22} = 2 Hz, 21-CH₂), 5.08 (m, 1 H, H-3), 6.01 (t, 1 H, J_{21,22} = 2 Hz, 21-CH₂); mass spectrum m/e 474 (M⁺).

Anal. Calcd for C₂₇H₃₈O₇: C, 68.33; H, 8.07. Found: C, 68.22; H, 8.15.

Compound **3b** was also obtained in the absence of 4-dimethylaminopyridine [41% (100% purity)], but the reaction time was 72 h.

18,20-Oxido-20,22-dihydrodigitoxigenin 3-Acetate from 3b. A solution of **3b** (1.01 g) in anhydrous methanol (80 mL) containing DBN (0.8 mL) was left at room temperature for 18 h. Saturated sodium chloride solution was added, and the product was extracted into ethyl acetate. The extract was washed to neutrality with water, dried over sodium sulfate, and evaporated in vacuo. The residue [0.84 g, 89%; $[\alpha]_D^{25} +21^\circ$ (c 0.255, CHCl₃); UV (MeOH) 218 nm (ϵ 490)] was further purified by TLC on silica gel [ethyl acetate-hexane (2:3)] and by crystallization from ethyl acetate-ether. The material obtained in this way had: mp 214–217 °C dec (softens at 205 °C); $[\alpha]_D^{25} +50^\circ$ (c 0.26, CHCl₃); UV (MeOH) 215 nm (ϵ 280); IR (CHCl₃) 3545, 1790, 1732 cm⁻¹.

Anal. Calcd for C₂₅H₃₆O₆: C, 69.42; H, 8.39. Found: C, 69.59; H, 8.43.

A small amount (0.075 g) of the alcohol **2g** was also obtained in this reaction.

Registry No. **1a**, 466-07-9; **1b**, 25633-33-4; **1d**, 25633-35-6; **1e**, 6859-20-7; **1e** acetate, 71129-70-9; (*R*)-**2a**, 71129-71-0; (*S*)-**2a**, 71183-76-1; (*R*)-**2b**, 71183-77-2; (*S*)-**2b**, 71129-72-1; (*R*)-**2c**, 71129-73-2; (*S*)-**2c**, 71183-78-3; (*R*)-**2d**, 71129-74-3; (*S*)-**2d**, 71183-79-4; (*R*)-**2e**, 71129-75-4; (*S*)-**2e**, 71183-80-7; (*R*)-**2f**, 71129-76-5; (*S*)-**2f**, 71183-81-8; (*R*)-**2g**, 71129-77-6; (*S*)-**2g**, 71183-82-9; (*R*)-**2h**, 71129-78-7; (*S*)-**2h**, 71183-83-0; (*R*)-**2i**, 71129-79-8; (*S*)-**2i**, 71183-84-1; **3b**, 71129-80-1; **3c**, 808-19-5.

Revised Assignment of the Electron Spin Resonance Spectrum Obtained by Photolysis of Tetrasulfur Tetranitride-Bis(norbornene)¹

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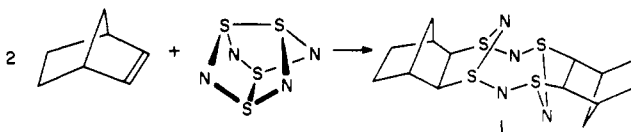
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Isotopic labeling with ³³S has been used to show that the radical obtained by photolysis of tetrasulfur tetranitride-bis(norbornene) and by the thermal reaction of tetrasulfur dinitride with norbornene is *exo*-1,2-norbornyl-1',3',2'-dithiazolidin-2'-yl. Several analogous radicals have been prepared and it is suggested that S₄N₄ adducts with unsaturated hydrocarbons may provide a starting point for the synthesis of compounds containing the 1,3,2-dithiazolidine ring system.

Becke-Goehring and Schläfer⁵ first reported that tetrasulfur tetranitride⁶ forms 1:2 adducts with norbornene and norbornadiene. The structure they proposed for these adducts⁵ and alternative structures suggested by Gleiter⁷ and by Brinkman and Allen⁸ have been shown to be incorrect.⁹⁻¹¹ For example, X-ray crystallographic studies^{9,10} of the norbornadiene adduct have shown that a C=C

double bond in each olefin adds across an S-N-S unit to give a five-membered C-S-N-S-C ring. Analogous structures appear to be formed with other strained olefins.¹¹ Thus norbornene will yield an adduct having structure **1**.



Sutcliffe and Brinkman^{12,13} have recently reported that photolysis of **1** in degassed solutions yields an extremely persistent radical X[•] which has a lifetime of a year or more (eq 1). Compounds analogous to **1** also gave persistent



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