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IRIDOID ALLOSIDES FROM *VIBURNUM OPULUS*

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Key Word Index—*Viburnum opulus*; Caprifoliaceae; opulus iridoids; acylated allosides; arbutin; ^{13}C-NMR spectra.

Abstract—Arbutin and four novel iridoid glycoside esters, named opulus iridoids I–IV, have been isolated from foliage of *Viburnum opulus* (Caprifoliaceae). Each opulus iridoid constitutes an inseparable mixture of two compounds, differing by containing either 2-methyl- or 3-methylbutyric acid in ester linkage at the 1-OH-group in an iridoid glycoside. In all glycosides 2′,3′-di-*O*-acetyl-β-D-allopyranose is linked through a glycosidic bond to C-11 in the iridoid aglycone. The opulus iridoids differ by the degree of acetylation of the aglycone and by the attachment, in III and IV, of a β-D-xylopyranosyl group at C-4 of the allose moiety. The structures have been elucidated by ^{1}H and ^{13}C-NMR spectroscopy and by cleavage of the glycosidic linkage with boron trifluoride etherate in acetic anhydride, yielding the acetates of the cyclized aglycone and of the appropriate mono- or disaccharide. This is the second report of an iridoid attached to a sugar other than glucose and the second time allose has been encountered in higher plants. The systematic position of *Viburnum* is briefly discussed.

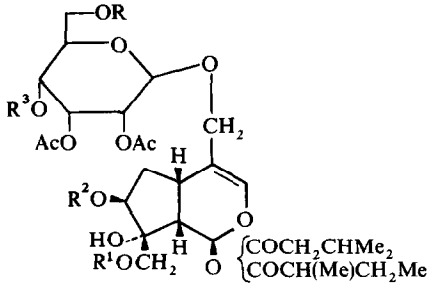
INTRODUCTION

Viburnum opulus (common snowball) is an ornamental shrub and extracts of the bark have been used as drugs [1]. A number of flavonoid glycosides have been reported from the flowers [2] and catechin from the bark [1]. Iridoids have been reported from *Viburnum* in two cases, adoxoside [3] from some species (*V. juddii*, *V. burkwoodii* and *V. dentatum*) and an iridoid pyrane from an acid hydrolyzed extract of *V. tinus* [4].

RESULTS AND DISCUSSION

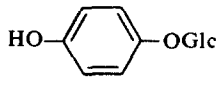
Extraction of fresh leaves gave an aqueous fraction containing a large number of glycosides as shown by TLC and PMR. The main constituents, arbutin (7) and four isomeric pairs of iridoid glycoside esters, which we have named opulus iridoid I, II, III and IV (structures **1–4**), were isolated by chromatography. Each pair of isomers represents an inseparable mixture of iridoid glycoside esters, containing 2-methylbutyric acid (of unknown enantiomeric composition) and 3-methylbutyric acid in an approximate ratio of 2:1. In the following discussion, such a mixture will be treated as a single compound.

The compounds were obtained as colourless syrups which turned yellow on standing and emitted an odour of isovaleric acid. The PMR spectra of the opulus iridoids all exhibited a broad singlet at *ca* 6.4 ppm and a doublet at *ca* 6.25 ppm, and this, combined with the odour of isovaleric acid, indicated structures of the 'Valeriana iridoid'-type [3], a group of iridoids esterified at C-1 with isovaleric acid, and containing an 11-CH_2OR function. The PMR spectrum of opulus iridoid I (**1**) showed 4 acetoxy groups (*ca* 2.1 ppm) and signals indicating that the compound was, in fact, a mixture of C_5-carboxylic esters (see Experimental). Acetylation provided a hexaacetate (**5**), demonstrating the presence of two esterifiable hydroxy groups in **1**. The ^{13}C-NMR spectrum of **1** showed apart from the acyl groups mentioned above, signals corresponding to 16 carbon atoms, consistent with an iridoid glycoside structure. Two absorptions, a doublet (J = 191 Hz) at 139.9 ppm and a singlet at 113.3 ppm could be assigned to C-3 and C-4, respectively, in an iridoid substituted at C-4. Doublets (J = 164 and 174 Hz) at 97.0 and 89.5 ppm could be assigned to C-1′ and C-1, respectively, on the the basis of the coupling constants [5], C-1′ being attached through a β-glycosidic linkage. Absorptions from C-1 in a variety of iridoids appear in the interval 92.6–103.6 ppm if C-1 is bound to glucose [5–9]. The high field shift (89.5 ppm) in the spectrum of **1** could be explained by the presence of an acyloxy group at C-1, as in patrinoside [10] and valerosidatum [11, 12]. Of the remaining twelve carbon atoms, nine carried oxygen (62–83 ppm). Five of these could be ascribed to the carbohydrate moiety



1 $R^1 = R^2 = Ac$; $R = R^3 = H$
2 $R^2 = Ac$; $R = R^1 = R^3 = H$
3 $R^1 = Ac$; $R = R^2 = H$; $R^3 = Xyl$
4 $R = R^1 = R^2 = H$, $R^3 = Xyl$
5 $R = R^1 = R^2 = R^3 = Ac$
6 $R = R^1 = R^2 = Ac$, $R^3 = XylAc_3$

* Part 2 in the series 'Glycosides in *Viburnum*'. For part 1 see Ref. 24.



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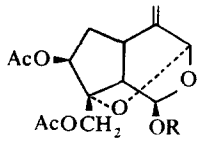
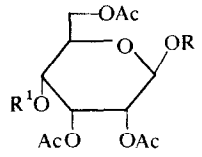
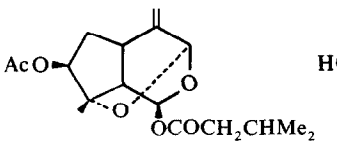


8 R = Ac
10 R = $MeCH_2CO$



9 R = R^1 = Ac
11 R^1 = Ac; R = $MeCH_2CO$
12 R = Ac; R^1 = $XylAc_3$



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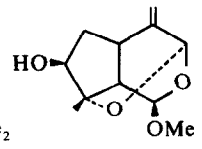


14

(4 × CH and 1 CH_2) and the remaining four to the aglycone, which should then have four oxygenated (1 C, 1 CH and 2 CH_2) and three non-functionalized carbons (2 CH and 1 CH_2)—in agreement with structure **1** and with the PMR spectra of **1** and **5**. Treatment of **5** with $BF_3(Et_2O)$ in Ac_2O at −18° cleaved the glycosidic linkage to give penta-*O*-*β*-D-allopyranoside (**9**) and the dioxatricyclodecane **8** formed by cyclization of the aglycone [13]. This established the nature of the sugar and the relative stereochemistry at C-5, C-8 and C-9 of the aglycone, as the rings in **5** must be *cis* fused and the 8-OH group α-oriented for the cyclization to proceed. The PMR spectrum of **8** greatly resembles those reported [12] for **13** and **14**, except for the differences expected from the presence of the 10-acetoxy group in **8**. Thus the coupling constants $J_{1,9}$, $J_{6\alpha,7}$ and $J_{6\beta,7}$ (3.5, 7 and 3.5 Hz) are the same as those reported for **13**, indicating the same relative stereochemistry at all chiral centres in **8**, **13** and **14**. The specific rotation found for **8** (+41°) is of the same sign and magnitude as those reported [12] for **13** and **14** (+71 and +49°, respectively), suggesting the same absolute configuration for the three compounds. To prove that the C_5-acyloxy group in **1** and **5** is located at C-1, cleavage of **5** was repeated using $BF_3(Et_2O)$ in $(EtCO)_2O$. This gave, as the only recognizable products, tetra-*O*-acetyl-*β*-D-allopyranoside monopropionate (**11**) and a dioxatricyclodecane (**10**) with two acetoxy groups and one propionyloxy group (PMR). Thus, only the acetalic carbon atoms (C-1 and C-1′) are attacked by the reagent under the conditions used. However, inversion might have occurred at C-1 and/or C-1′ during cleavage. Application of the NMR shift reagent Eu(fod)$_3$ in a series of PMR spectra of **5** proved that the protons at C-1 and C-7 were indeed situated on the same side of the molecule—indicating that inversion had not occurred

Table 1. PMR data for the *Viburnum opulus* iridoids. (90 MHz; for further details, see Experimental)

Compound	H-1	H-3	H-5	H-6	H-7	H-9	H-10	H-11	H-1′ H-1″	H-2′ H-2″	H-3′ H-3″	H-4′ H-4″	H-5′ H-5″	H-6′ H-5″	Number of AcO groups
1	6.3	6.45	2.94	*ca* 2	5.02	2.45	4.24	4.20	—4.8—		5.6	— 3.6–4.0 —			4
	d(4)	*br s*	*m*		*t*(*ca* 3)	*dd*(4, 9)	*br s*	AB(13)			*br t*(*ca* 3)				4
2	6.18	6.42	2.9	*ca* 2	4.93	2.42	3.62	4.19	—4 8—		5.60	—3.6–4.0—			
	d(5)	*br s*	*m*		*t*(*ca* 4)	*dd*(5, 9)	*br s*	*br* AB(13)			*br t*(*ca* 3)				3
3	6.2	6.38	2.91	*ca* 2	*ca* 4.8	2.49	4.26	4.26	4.99	4.75	5.73	— 3.1–4.1 —			
	d(2)	*br s*	*m*			*dd*(2, 10)			*d*(8)		*br t*(3)				
									4.48		— 3.1–4.1 —				3
									d(7)						
5	6.29	6.42	2.89	*ca* 2	5.04	2.44	4.3	4.2	—4.9—		5.67	4.9	—*ca* 4.3—		
	d(4)	*br s*	*br t*(9)		*br t*(*ca* 2)	*dd*(4, 9)	*br s*	AB(12)			*t*(3)				6
6	6.27	6.39	2.88	*ca* 2	*ca* 5.0	2.42	4.25	4.2	—4.8—		5.72	3.8	—*ca* 4—		
	d(4)	*br s*	*br t*(*ca* 5)			*dd*(4, 9)	*s*				*br t*(3)	*dd*(3, 10)			8
									4.56		—4.8–5.3—			3.35	
									d(6)					*dd*(8, 12)	
8	6.43	5.23	3.28	2.31	5.15	2.65	4.56								
	d(3)	*s*	*br t*(*ca* 5)	*dd*(7, 14)	*dd*(3, 7)	*dd*(3, 4)	4.28								
				1.95			AB(12)								3
				ddd(3, 6, 14)											
9									6.07	5.0	5.72	5.0	— 4.25 —		
									d(8)	*m*	*t*(3)	*m*			5
12									5.93	4.98	5.76	3.80	—*ca* 4 1—		
									d(8.5)	*dd*(3, 8)	*t*(3)	*dd*(9, 13)			7
									4.54		—4.8–5.25—		4.03	3.33	
									d(6.5)				*dd*(4, 12)	*dd*(8, 12)	

Table 2. ^{13}C-NMR data for the *Viburnum opulus* iridoids*

Compound†	1	2	3	4	5	6	9	β-D-allopyranose	Methyl β-D-allopyranoside Ac$_4$	Methyl β-D-xylopyranoside	Methyl β-D-xylopyranoside Ac$_3$	12	10	15	16	17
C-1	89.5 d(174)	90.0 d	91.3	91.4 d(177)	89.4 d(171)	89.4							89.7 d(179)	92.6 d(176)	88.4 d(174)	90.0
C-3	139.9 d(191)	139.8 d	139.8	139.6 d(191)	140.2 d(189)	140.2							93.7 d(179)	148.6 d(191)	142.2 d(190)	139.7
C-4	113.3 s	113.7 s	115.8	115.8 s	113.0 s	113.1							147.1 s	108.4 s	110.9 s	108.3
C-5	31.9 d(130x)	32.3 d	30.7	31.0 d(134)	32.0 d(135x)	32.0							36.9 d(143x)	140.9 s	32.6 d(135x)	31.5
C-6	34.8 t(133x)	34.8 t	36.9	37.1 t(130)	34.7 t(131x)	34.7							40.7 dd(136, 130)	118.7 d(173)	35.1 d(130x)	37.2
C-7	80.5 d(158x)	80.5 d	78.6	78.6 d(150)	80.6 d(154)	80.5							77.9 d(163)	83.1 d(158)	76.8 d(155)	77.5
C-8	81.1 s	82.3 s	82.3	83.6 s	81.0 s	81.1							81.9 s	64.2 s	64.2 s	82.9
C-9	44.8 d(131)	43.9 d	44.5	44.0 d(131)	44.8 d(133)	44.8							41.0 d(141)	43.0 d(136)	39.5 d(137)	43.7
C-10	67.0 t(146)	64.2 t	68.5	65.2 t(142)	67.0 t(148)	66.9							64.4 t(149)	47.8 t(176)	48.8 t(178)	66.4
C-11	68.6 t(143)	68.4 t	70.0	70.0 t(145)	68.5 t(141)	68.7‡							109.3 t(158)	60.8 t(149)	63.3 t(149)	63.5
C-1′	97.0 d(164)	97.0 d	97.8	97.8 d(158x)	97.0 d(163)	96.8	90.1 d(165)	94.3 d(161)	99.3 d(163)			89.9				
C-2′	70.2 d(148)	70.0 d	71.2	71.1 d(155x)	69.2 d(150)	69.6	68.2 d(155)	72.2 d(142)	68.9 d(150)			68.9				
C-3′	71.4 d(156)	71.1 d	71.2	71.1 d(155x)	68.5 d(159)	69.9	68.2 d(155)	72.0 d(148)	68.2 d(160)			69.4				
C-4′	66.2 d(145)	65.7 d	74.5‡	74.2 d(143x)	66.4 d(146)	74.1	65.8 d(145)	67.7 d(145)	66.1 d(147)			73.6				
C-5′	74.1 d(142x)	73.9 d	74.2‡	74.2 d(143x)	70.2 d(145)	70.8	71.2 d(148)	74.4 d(150)	70.0 d(142)			71.6				
C-6′	62.2 t(143)	62.1 t	61.1	61.2 t(144)	62.4 t(147)	62.4‡	61.9 t(149)	62.1 t(145)	62.1 t(149)			62.5				
C-1″			104.7	104.7 d(158x)		101.8				104.8 d(159)	101.0 d(161)	102.0				
C-2″			73.8	73.8 d(145)		71.0				73.9 d(144)	70.2 d(153)	71.0				
C-3″			76.5	76.6 d(145)		71.6				76.7 d(144)	71.0 d(152)	71.6				
C-4″			70.0	70.0 d(147)		68.9‡				70.1 d(147)	68.3 d(153)	68.9				
C-5″			66.0	66.0 dd(143, 152)		62.7‡				66.0 dd(142, 150)	61.3 dd(143, 151)	62.5				

* The spectra were recorded as earlier described [5]; solvent: $CDCl_3$, except for **3** and **4**, where D_2O was used. Chemical shifts in ppm ± 0.1. Coupling constants in Hz ± 2 Hz (if the value is followed by an 'x': ± 5 Hz).

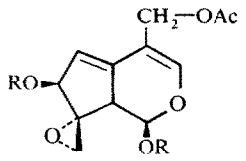
† The compounds **1–6** all had additional signals arising from acetoxy groups at *ca* 170 and 20.5 ppm, from 2-methylbutyroyl groups at *ca* 177(*s*), 41(*d*), 27(*t*), 16.5(*q*) and 11.5(*q*) ppm and 3-methylbutyroyl groups at *ca* 173(*s*), 43(*d*), 25.5(*t*) and 22(*q*) ppm. Compounds **15–17** all showed absorptions from the latter moiety, and **16** in addition those from an acetoxy group.

‡ Interchangeable in the same vertical column.

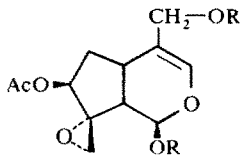
during the cleavage of **5**. At the same time it was shown that **5** was a β-alloside. The coupling constant $J_{1',2'}$ not seen in the original spectrum of **5** (or **1**) due to higher order coupling effects, was revealed by the shift reagent to be 9 Hz, confirming the result found above using ^{13}C-NMR data. Only the positions of the four acetoxy groups in **1** remained to be settled. Comparison of the ^{13}C-NMR spectrum of **5** with that of methyl β-D-allopyranoside tetraacetate (see Table 2) showed very good correspondence for six of the absorptions arising from **5**, and allowed distinction with certainty between the signals arising from the sugar moiety and those from the aglycone. The spectra of **1** and **5** differed only in signals from the sugar parts of the molecules, showing that acetylation of **1** had occurred there. Signals at 5.6 and 4.32 ppm (2H) in the PMR spectrum of **1** could be assigned to the protons of C-3′, C-1′ and C-2′, respectively, and further decoupling experiments revealed the C-4′ proton at *ca* 3.8 ppm. The low field shifts of the C-2′ and C-3′ protons showed that these were acetylated; therefore the structure of opulus iridoid I must be **1**, with acetoxy groups at the positions 7, 10, 2′ and 3′.

Opulus iridoid II (**2**) contained three acetoxy groups as seen from the NMR spectra. Acetylation provided the same hexaacetate (**5**) as that obtained from **1**. In the PMR spectra of **1** and **2**, the signals arising from the sugar moiety had virtually the same shifts in the two sets, suggesting that the difference between **1** and **2** resided in the aglycone part. This was proven by the ^{13}C-NMR spectra of **1**, **2** and **5**, correct assignment being secured by selective decoupling experiments, use of expected shift positions together with the multiplicities seen in the proton coupled spectra, and comparison with spectra of other compounds [5–9]. Comparison between **1** and **2** showed that only the chemical shifts of C-8, C-9 and C-10 were different, the former showing a downfield shift from **1** to **2** of 1.2 ppm, and the other two upfields shifts (0.9 ppm and 2.8 ppm), respectively. This is only consistent with a free OH-group at C-10 in **2**. In opulus iridoid II, therefore, the positions 7, 2′ and 3′ are acetylated.

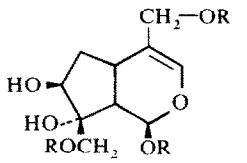
Opulus iridoid III (**3**) and IV (**4**) contained, according to PMR, three and two acetoxy groups, respectively. Acetylation of both provided the same octaacetate (**6**). Treatment of **6** with $BF_3(Et_2O)$ as above gave **8** together



15



16 R = $COCH_2CHMe_2$



17

with a disaccharide heptaacetate (**12**). The ^{13}C-NMR spectra of **5** and **6** exhibited exactly the same pattern for the aglycones of the two compounds. Again, the spectrum of the allose moiety of **5** was rather similar to that of the corresponding moiety of **6**, apart from a small change of the signal of C-3′ (+ 1.4 ppm) and a large change of that of C-4′ (+ 7.7 ppm), consistent with the assumption that the second carbohydrate moiety was linked to the latter carbon atom. The remaining five signals in the spectrum of **6**, not corresponding to any in the spectrum of **5**, indicated that the extra carbohydrate moiety of **6** was a pentosyl group.

Comparison of this part of the spectrum with that of methyl-β-D-xylopyranoside triacetate [14] (see Table 2) showed, except for a general deviation of *ca* 0.7 ppm, very close agreement. A similar agreement was observed also for five signals in the spectrum of the disaccharide heptaacetate (**12**) isolated from the cleavage experiment. This suggested that the glycone of **3**, **4** and **6** might, disregarding the acetoxy groups, be 4-*O*-(β-xylopyranosyl)-β-allopyranose, a supposition supported by the PMR data. Thus, in the spectrum of the disaccharide heptaacetate **12**, two sets of signals could be discerned. One set was, except for the expected small downfield shift (0.18 ppm) of the proton at C-1, almost superimposable on a spectrum of methyl-β-D-xylopyranoside triacetate, and decoupling experiments showed the expected coupling constants. The other set partly matched with a spectrum of β-D-allopyranoside pentaacetate [14], showing the same coupling pattern, but with a large upfield shift (1.2 ppm) of the signal arising from the proton at C-4. Thus the information from ^{1}H and ^{13}C-NMR spectroscopy confirms that the relative stereochemistry of **12** conforms with that of 4-*O*-(β-xylopyranosyl)-β-allopyranose. That inversion at C-1′ had not taken place by the cleavage of **6** was seen from the large coupling constant (8 Hz) exhibited by the proton at C-1′ in the PMR spectrum of both **3** and **4**, proving the same sterochemistry at this centre in **3**, **4**, **6** and **12**. Assuming D-stereochemistry for the allose moiety in the disaccharide, as found in **1** and **2**, application of Klyne's rule [15, 16] indicates that the xylose unit of **12** also has D-configuration. Thus the molecular rotation of penta-*O*-acetyl-β-D-allopyranose is $[M]_D$ −62° and that of methyl tri-*O*-acetyl-β-D-xylopyranoside is $[M]_D$ −176°. The measured $[M]_D$ of **12** is −188°, strongly indicating that this compound is hepta-*O*-acetyl-4-*O*-(β-D-xylopyranosyl)-β-D-allopyranose.

The only remaining question to be settled concerns the positions of the acetoxy groups in **3** and **4**. In the PMR spectra of both compounds, the absorptions arising from the protons at C-2′ and C-3′ are found at the same shift as in the common peracetate **6**, proving that these positions are acetylated in both compounds. The remaining acetoxy group of **3** must then be placed at C-10, because only the protons at this position are shifted (*ca* 0.45 ppm upfield) when going from **3** to **4**. The ^{13}C-NMR spectra are in agreement with this conclusion.

In Table 2, we have included the ^{13}C-NMR spectrum of valtratum (**15**), didrovaltratum (**16**) and a compound (**17**), derived from **16** [11]. Of interest is the change seen by comparing the spectra of the 8, 10-dioxygenated compounds with those of the 8, 10-epoxy compounds **15** and **16**, the two carbon atoms showing an upfield shift of *ca* 18 ppm in the latter compounds. The large C-H coupling constants of the epoxide carbon atoms, *ca* 175 Hz, compared with the normal value, *ca* 150 Hz, appears to be a promising tool in assignment of carbon atoms carrying epoxide functions. The remaining carbon absorptions correspond closely to the values found for the opulus iridoids, allowing for differences in functionality.

Extracts from samples collected during the summer contained mainly **1**, whereas material collected in October contained mainly **2**, **3** and **4**.

This is the second report on the occurrence of sugars other than glucose as constituents of iridoid glycosides, the first being the compound montinioside from *Montinia caryophyllaceae* containing both glucose and xylose [17]. Allose has only once been reported as a constituent of higher plants, namely as part of phenolic glycosides isolated from *Protea rubropilosa* Beard [18].

The odour of isovaleric acid from some species of *Viburnum* has been noted [19, 20, 21], and, partly because of this, a close relationship has been supposed between *Viburnum* and the Valerianaceae. The present finding of iridoid glycosides of the same type as the Valeriana compounds supports Hallier's view. The opulus iridoids are rather different from those found in *Sambucus* [22] and in Caprifoliaceae *sensu strictu* (loganin and secoiridoids [3, 9]) suggesting that the relationship between these taxa and *Viburnum* is not very close.

Crude extracts of a large number (*ca* 50) of species of *Viburnum* have so far been examined. Iridoid glycosides of different types have been detected in about half of the species. Compounds resembling the opulus iridoids were most commonly encountered.

EXPERIMENTAL

General procedures were as earlier described [5, 23]. Microanalyses were performed at NOVO Microanalytical Laboratory, Bagsværd, Denmark. Foliage of *Viburnum opulus* was collected in June 1972 at Sjællands Odde (IOK-116/72) and at Hornbæk Plantage (IOK-119/72). Also in October 1975 and August 1977 at Holte (IOK-103/75). It was kept at −23° until use. The vouchers have been deposited at the Botanical Museum, University of Copenhagen, Denmark.

Isolation of the opulus iridoids. Frozen leaves (760 g) collected in October were homogenized in EtOH, filtered and evapd to near dryness, after which the extract was partitioned between H_2O and $CHCl_3$. The aq. layer was worked up as earlier described [23], to give *fraction B*, (10 g). The $CHCl_3$-layer was evapd to dryness and partitioned between pentane (500 ml) and MeOH–H_2O (2.5:1, 500 ml). Concn of the latter gave a brown syrup

(2.2 g), which was redissolved in MeOH and filtered through charcoal. Evapn provided a clear syrup (1.9 g, *fraction A*). TLC showed several spots and chromatography on Si gel (300 g) with $CHCl_3$–MeOH (8:1 to 3:1) as eluents yielded as the first fraction **1** (230 mg, 0.03%) containing small amounts of **2**. Plants collected in the summer all had opulus iridoid I (**1**) as the major compound. Thus material from the same plant in August 1977 contained *ca* 0.3% of **1**. The collections from June (119/72 and 116/72) gave 0.73 and 0.3% **1**, respectively. Rechromatography and treatment with activated carbon gave the analytical sample, $[\alpha]_D^{19}$ −64° (*c* 1.1; $CHCl_3$). (Found: C, 53.88; H, 6.68; $C_{29}H_{42}O_{16}$ requires: C, 53.85; H, 6.56%). The next fraction (360 mg), which consisted of almost pure **2**, was rechromatographed and treated with activated carbon to give the analytical specimen of opulus iridoid II $[\alpha]_D^{20}$ −55° (*c* 2.3, MeOH); PMR data: signals arising from the 2-methyl-butyroyl group: 1.66 ppm (*p*, *J* = 8 Hz, CH_2), 1.17 ppm (*d*, *J* = 7 Hz, α-Me) and 0.94 ppm (*t*, *J* = 8 Hz, β-Me); signals from the 3-methyl-butyroyl group; 0.99 ppm (*d*, *J* = 7 Hz, 2 × Me). The remaining signals from these acyl groups could not be discerned. This pattern was consistent through the spectra of the 'compounds' **1**–**6**, the ratio of 2-methylbutyroyl: 3-methylbutyroyl being *ca* 2:1. (Found: C, 52.25; H, 6.66; $C_{27}H_{40}O_{15}$ requires: C, 51.93; H, 6.46%). The remaining fractions from A were mixtures and were not further investigated. TLC of fraction B showed the presence of several compounds. Chromatography as above of an aliquot (3.8 g) gave a further amount (140 mg, total 0.07%) of **2**. After some fractions containing mixtures (*ca* 600 mg), the next pure component (240 mg, 0.08%) was eluted. Treatment with activated carbon gave the analytical specimen of opulus iridoid III (**3**) as a colourless glass. (Found: C, 50.51; H, 7.06. $C_{32}H_{48}O_{19}$, $1\frac{1}{2}$ H_2O requires: C, 50.33; H, 6.73%). The next fraction (360 mg) was rechromatographed by PLC to give as the faster running band opulus iridoid IV (**4**, 160 mg, 0.02%). (Found: C, 49.41; H, 6.88. $C_{30}H_{46}O_{18}$, 2 H_2O requires: C, 49.26; H, 6.67%). The slower moving band yielded arbutin (**7**, 160 mg, 0.02%), mp and mmp 200°.

Acetylation of **1** *or* **2**. (Py, Ac_2O, 4 hr) gave **5**, $[\alpha]_D^{17}$ −58° (*c* 3.7 $CHCl_3$). (Found: C, 54.04; H, 6.32; $C_{33}H_{16}O_{18}$ requires: C, 54.24; H, 6.35%).

Cleavage of **5**. To 650 mg **5** in Et_2O–Ac_2O (1:1, 9 ml) at ÷18° was added $BF_3(Et_2O)$ (1.5 ml) under stirring. After 15 min ice and $CHCl_3$ was added and the organic phase separated, washed with $NaHCO_3$ soln and dried. Evapn of the solvent gave a crude syrup (709 mg), which was chromatographed by PLC (Si gel; pentane–Me_2CO, 2.5:1) to give as the faster moving band **8** (167 mg) $[\alpha]_D^{21}$ +41° (*c*, 2.4; $CHCl_3$). (Found: C, 56.24; H, 5.84); $C_{16}H_{20}O_8$ requires: C, 56.46; H, 5.92%). From the slower moving band was obtained penta-*O*-acetyl-β-D-allopyranose (**9**, 257 mg); mp and mmp 99.5–100°; $[\alpha]_D^{21}$ −16° (*c*, 0.9; $CHCl_3$). The cleavage of **5** was repeated with propionic anhydride. Thus **5** (375 mg) was treated as above to give **10** (113 mg), mp 97°; $[\alpha]_D^{18}$ +63° (*c* 0.4; $CHCl_3$); PMR data: 2.59 ppm (*q*, *J* = 7.5 Hz, CO—CH_2), *ca* 2 ppm (2 *s*, 2 × AcO), and 1.14 ppm (*t*, *J* = 7.5 Hz, Me). (Found: C, 57.40; H, 6.14; $C_{17}H_{24}O_{11}$ requires: C, 57.61; H, 6.26%). The second fraction was 1-*O*-propionyl-2,3,4,6-tetra-*O*-acetyl-β-D-allopyranose (**11**, 105 mg), mp 82–82.5°, $[\alpha]_D^{20}$ −16° (*c*, 0.25; $CHCl_3$). The PMR spectrum was identical to that of **9**, except for the exchange of one acetyl for one propionyl group. (Found: C, 50.72; H, 6.06; $C_{17}H_{24}O_{11}$ requires: C, 50.50; H, 5.98%).

Acetylation of **3** *or* **4** as above provided **6**, an octaacetate, as a colourless syrup, $[\alpha]_D^{18}$ −52° (*c*, 0.3; $CHCl_3$). (Found; C, 53.91; H, 6.22; $C_{42}H_{58}O_{24}$ requires: C, 53.28; H, 6.18%).

Cleavage of **6**. 670 mg **6** as above (Ac_2O) gave **8** (107 mg), and **12** (220 mg), mp (Et_2O) 147–148°, $[\alpha]_D^{20}$ −31° (*c*, 0.3; $CHCl_3$). (Found: C, 49.56; H, 5.75; $C_{25}H_{34}O_{17}$ requires: C, 49.50; H, 5.65%).

Acetylation of a crude mixture. Fraction B, (1.85 g) treated as above gave, after chromatography (Si gel, C_6H_6–EtOAc, 1:1) three fractions. Fraction 1 (210 mg) consisted mainly (PMR) of arbutin pentaacetate with small amounts of sugar acetates. Fractions 2 (390 mg) and 3 (550 mg) consisted of pure **5** and **6** respectively.

Identification of 2-methyl- and 3-methylbutyric acid. Performed by adding NaOMe to a soln of **1** in MeOH. GLC (SCOT Carbowax, 70°) showed two peaks corresponding to authentic methyl esters; methyl 2-methylbutyrate (MS: *m/e* 101, 88, 85, 57, 41) and methyl 3-methylbutyrate (MS: *m/e* 101, 85, 74, 59, 57, 43, 41).

Application of PMR shift reagent. Performed by dissolving **5** (*ca* 40 mg) in $CDCl_3$ and adding successively 11, 10 and 20 mg $Eu(fod)_3$. A linear plot was obtained for all absorptions, H-7, H-10, H-11 (!) and H-1 shifting *ca* 1.8 ppm downfield, while H-9 shifted 1.2 ppm, H-5 shifted 0.8 ppm and H-3 shifted 0.5 ppm.

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