piperazine indicated that only 16.4% of *N*-methylpiperazine could be recovered under these conditions. Hydrolysis of DIP in neutral solution thus leads to *N*-formyl-*N'*-methylpiperazine and *N*-methylpiperazine in stoichiometric and equivalent amounts. Authentic *N*-formyl-*N'*-methylpiperazine was synthesized by the reaction of methyl formate with *N*-methylpiperazine, followed by purification with column chromatography. The liquid obtained in this way gives a single spot on TLC and has an NMR spectrum in complete accord with its assigned structure: NMR ($CDCl_3$) δ 2.32 (3 H, s), 2.45 (4 H, t), 3.50 (4 H, q), 8.05 (1 H, s); ir 1660 cm^{-1} (C=O).

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Polyglucosidic Metabolites of Oleaceae. The Chain Sequence of Oleoside Aglucon, Tyrosol, and Glucose Units in Three Metabolites from *Fraxinus americana*

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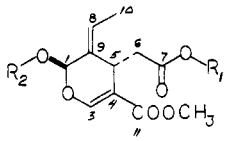
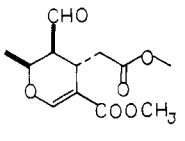
Abstract: Metabolites, previously designated Gl 3, Gl 5, and Gl 6, from embryos of the American ash (*Fraxinus americana*) were examined by spectral methods, degradation achieved by methanolysis and conversion to acetates. Gl 6 is identified as nüzhenide. Acetylation of Gl 3 and Gl 5 gives ^1H NMR determined undecaacetate and octaacetate, respectively, thus providing evidence for the number of free hydroxyl groups and furnishing material suitable for molecular weight determinations by vapor-phase osmometry. Evidence for the identity and sequence of the various units was obtained, among other methods, through methanolysis, Gl 3 giving nüzhenide and oleoside 7-methyl ester and Gl 5 giving ligstroside and oleoside 7-methyl ester. Gl 3 contains one unit of 2-(4-hydroxyphenyl)ethanol (Tyo), three units of glucose [all as 1-β-D-glucopyranosides (β-D-Glc)], and two of oleoside aglucon (β-Olo) in the sequence: β-D-Glcl-1β-Olo7-6β-D-Glcl-1Tyo6-7β-Olol-1β-D-Glc. Gl 5 contains the same components as Gl 3 but one less glucose unit in the sequence β-D-Glcl-1β-Olo7-1Tyo6-7β-Olol-1β-D-Glc. ^{13}C NMR proves to be an especially valuable tool in determining the sequence of units in the intact metabolites.

Sondheimer and co-workers[1] have described the discovery of three abundant glucosides, designated simply Gl 3, Gl 5, and Gl 6, from the seeds of *Fraxinus americana*. Besides the genus *Fraxinus*, the glucosides were detected in the seeds from the genera *Olea* and *Syringa*, also of the family Oleaceae. These workers observed that the levels of metabolites Gl 3 and 6, but not Gl 5, diminished in the course of germination and were regulated by gibberellic and abscisic acids. The nature of Gl 3, Gl 5, and Gl 6 remained obscure despite an earlier investigation of Gl 3.[2] This paper describes our efforts to elucidate the structures of all three metabolites.

The earlier examination[2] of Gl 3 gave evidence for the incorporation of the following structural features. Spectral data indicated the presence of -OCH=C(C)COOR in Gl 3. Acid-promoted hydrolysis gave glucose but the results to establish its presence in a β-glucoside linkage were equivocal.

Also isolated from the acid hydrolysis was what appears to be a mixture of at least two compounds, one of which contained an aldehyde group and another most likely was oleoside aglucon (**1**) since on treatment of the mixture with diazomethane, the spectral properties, particularly the ^{1}H NMR, indicated to us the presence of oleoside aglucon methyl ester (**2**) in the resulting mixture. We ascribed the origin of the aldehyde containing material to the presence of methyl elenolate (**3**),[3] formed from oleoside aglucon by acid-promoted rearrangement and the diazomethane treatment. Therefore the apparent degradation of Gl 3 to oleoside aglucon and elenolic acid strongly suggested the involvement of the structural unit -OCH=C(C)COOR in an oleoside moiety present in the intact Gl 3. Among the products of the base-promoted hydrolysis of Gl 3 was 2-(4-hydroxyphenyl)ethyl β-D-glucopyranoside (salidroside, **4**).



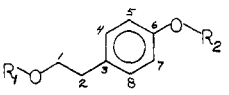


1, R_1=R_2=H(β-Olo)

2, R_1=CH_3; R_2=H

7, R_1=CH_3; R_2=1β-D-Glucopyranose

3



4, R_1=1β-D-Glucopyranose, R_2=H

5, R_1=R_2=H (Tyo)

11, R_1=CH_3CO, R_2=H

12, R_1=R_2=CH_3CO

13, R_1=H, R_2=CH_3CO

Thus the earlier work furnished evidence for the presence of three distinct moieties in Gl 3: glucose (β-D-Glc), oleoside aglucon (**1**, β-Olo), and 2-(4-hydroxyphenyl)ethanol (**5**, tyrosol [Tyo]). This assessment of the involved structural moieties served as a working hypothesis from which our studies were planned and executed.

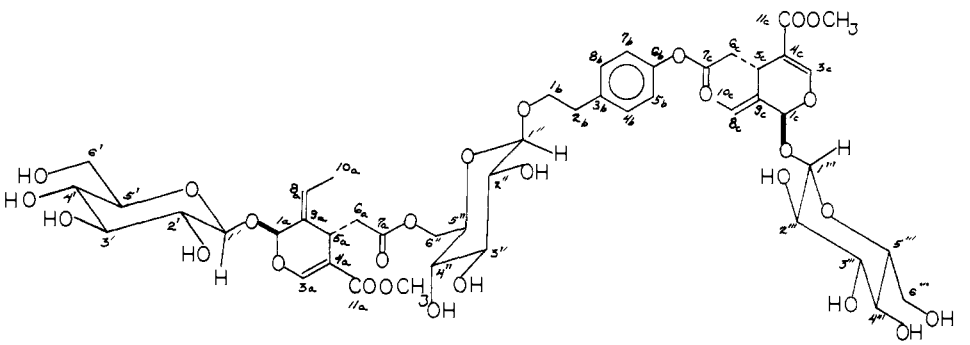
Gl 3 and Gl 6. The ^{1}H NMR spectra of Gl 3, **6**, and other Oleaceae glucosides and their acetates are summarized in Table I. Two methoxyl, two vinyl (3a and 3c H), and two acetalcarbinyl (1a and 1c H) signals were observed for Gl 3 but only one of each of these three different signals was observed in the spectrum of Gl 6, formed from Gl 3 on methanolysis (vide infra). Assuming that each vinyl and acetalcarbinyl signal represented a single proton, four aromatic protons (4b, 5b, 7b, 8b H) could be observed. Thus, using the sum of six vinyl (3a and 3c H) and aromatic protons (4b, 5b, 7b, 8b H) as a standard for integration, the presence of 11 acetylmethyls could be ascertained in Gl 3 acetate, which was prepared from Gl 3 by a conventional procedure. Therefore incorporated into the structure of Gl 3 were three glucose units, two of which were singly bound and thereby terminal units and a third doubly bound and thereby an internal unit. The original assumption regarding the number of protons assignable to vinyl and acetalcarbinyl signals meant that two oleoside aglucon, one tyrosol, and three glucose units were involved. Both the elemental analysis and the observed molecular weight of 1603 ± 23 for Gl 3 undecaacetate were consistent with this original assumption. The elemental analysis was determined on the undecaacetate rather than the glucoside since the latter gave a poorly defined hydrate.

Gl 3 gave no immediate color with ferric chloride, but on standing or when first treated with aqueous sodium hydroxide, a color was observed. These observations meant that the tyrosol unit was not involved as a free phenolic terminus.

Additional information pertinent to the sequence of units and evidence confirming the composition were obtained through the careful methanolysis of Gl 3 carried out at 80°. Oleoside 7-methyl ester (**7**), salidroside (**4**), and Gl 6 were formed in the ratio of 1.35:0.27:0.39. The last named proved identical with an authentic sample of nüzhenide (**8**).[4] Salidroside was also identified by comparison to an authentic sample while oleoside 7-methyl ester was converted to its tetraacetate which was identified by comparison to reported properties.[4] Since the phenolic oxygen of tyrosol was not present in Gl 3 as a phenolic function, the second oleoside residue must be attached through an ester linkage to C-6b in the aromatic ring. The preferential methanolysis of C-7c rather than C-7a, as was manifest in the ratio of methanolysis products, could be attributed to the better leaving ability of phenoxide as opposed to alkoxide. That the ester linkages are through C-7a and C-7c, rather than the C-11 centers, is proposed because of the usual greater ease with which saturated esters undergo solvolysis compared to α,β-unsaturated esters. As a case in point, both nüzhenide and Gl 3 undergo hydroxide promoted hydrolysis giving α,β-unsaturated methyl esters, i.e., leaving the methyl ester untouched. Because our methanolysis was carried out under even less drastic conditions than the aforementioned hydrolysis, C-11 linkages would be expected to survive had they been involved with C-6″ of glucose or C-6b of the tyrosol unit.

Evidence supporting structure **6** for Gl 3 was obtained from the ^{13}C spectrum and a correlation of these spectral properties with those of other Oleaceae metabolites. All the ^{13}C NMR spectral results are discussed together in a section to follow.

Gl 5. The ^{1}H NMR was like that of Gl 3 in showing two



6, β-D-Glc1-1 β-Olo7-6 β-D-Glc1-1 Tyo6-7 β-Olo1-1 β-D-Glc

8, β-D-Glc1-1 β-Olo7-6 β-D-Glc1-1 Tyo

9, β-D-Glc1-1 β-Olo7-1 Tyo6-7 β-Olo1-1 β-D-Glc

10, β-D-Glc1-1 β-Olo7-1 Tyo

Table I. Proton NMR Chemical Shifts (δ)[a] of Secoiridoid Glucosides and Their Acetates

Secoiridoid glucoside	Protons in the moiety											
	Oleoside aglucon (carbon no.)						Tyrosol (carbon no.)			Glucose (carbon no.)		
	1a, 1c	3a, 3c	5a	6a, 6a'	8a, 8c	10a, 10c	1b	2b	4b, 5b, 7b, 8b[b]	1	OCH_3	CH_3COO
Gl 3	5.87 (br s) 6.00 (br s) (2 H)	7.56 (s), 7.64 (s) (2 H)			6.09 (q, 7 Hz), 6.21 (q, 7 Hz) (2 H)	1.68 (d, 7 Hz), 1.74 (d, 7 Hz) (6 H)			6.95, 7.03, 7.28, 7.37 (4 H)	4.93 (d, 7.5 Hz) (1 H),[e] 4.47 (d, 7.5 Hz) (1H),[f] 4.93 (d, 7.5 Hz) (1 H)[g]	3.66[c] (s), 3.75 (s) (6 H)	
Gl 3 undecaacetate	5.75 (br s), 5.84 (br s) (2 H)	7.49 (s), 7.53 (s) (2 H)			6.03 (q, 7.5 Hz), 6.13 (q, 7.5 Hz) (2 H)	1.78 (dd, 7.5, 1.5 Hz), 1.78 (dd, 7.5, 1.5 Hz) (6 H)			6.89, 7.03, 7.15, 7.28 (4 H)	4.51 (d, 7.5 Hz) (1 H)	3.73[c] (s), 3.76 (s) (6 H)	1.91 (s), 1.98 (s), 2.02 (s), 2.07 (s) (33 H)
Gl 5	5.79 (br s), 5.96 (br s) (6 H)	7.49 (s), 7.60 (s) (2 H)			6.06 (q, 7 Hz), 6.14 (q, 7 Hz)	1.41 (d, 7 Hz), 1.69 (d, 7 Hz) (6 H)			6.95, 7.03, 7.25, 7.33 (4 H)	4.85 (d, 7 Hz), 4.86 (d, 7 Hz) (2 H)	3.70[c] (s), 3.72 (s) (6 H)	
Gl 5 octaacetate	5.66 (br s), 5.76 (br s) (2 H)	7.38 (s), 7.43 (s) (2 H)			5.93 (q, 7 Hz), 6.04 (q, 7 Hz) (2 H)	1.70 (dd, 7, 1.5 Hz), 1.79 (dd, 7, 1.5 Hz) (2 H)			6.83, 6.97, 7.07, 7.20 (4 H)		3.69[c] (s), 3.71[c] (s) (6 H)	1.99, 2.03 (24 H)
Nüzhenide (Gl 6)	5.84 (br s), (1 H)	7.54 (s) (1 H)			6.06 (q, 7 Hz) (1 H)	1.68 (dd, 7, 1 Hz) (3 H)			6.76, 6.85, 7.11, 7.20, (4 H)	4.89 (d, 7 Hz) (1 H),[e] 4.46 (d, 7 Hz) (1 H)[f]	3.69[c] (s) (3 H)	
Ligstroside	5.79 (br s) (1 H)	7.54 (s) (1 H)			6.04 (q, 7 Hz) (1 H)	1.60 (dd, 7,1 Hz) (3 H)			6.83, 6.91, 7.15, 7.24, (4 H)	4.89 (d, 7 Hz) (1 H)[e]	3.76[c] (s) (3 H)	
Salidroside							3.97 (d, 7 Hz) (2 H)	2.88 (t, 7 Hz) (2 H)	6.82, 6.90, 7.13, 7.20 (4 H)	4.45 (d, 8 Hz) (1 H)[f]		
Salidroside tetraacetate								2.72 (t, 6 Hz) (2 H)	6.82, 6.94, 7.17 (4 H)	4.42 (d, 7.5 Hz) (1 H)[f]		1.97 (12 H)
Oleoside 7-methyl ester	5.90 (br s) (1 H)	7.56 (s) (1 H)		2.0–3.0 (d of AB, 9.0, 5, 14.5 Hz) (2 H)	6.11 (q, 7 Hz) (1 H)	1.69 (dd, 7, 1 Hz) (3 H)				4.92 (d, 7.5 Hz) (1 H)[f]	3.72[c] (s) (3 H), 3.64 (s) (3 H)[d]	
Oleoside methyl ester tetra-acetate	5.72 (br s) (1 H)	7.46 (s) (1 H)	4.02 (dd, 5.1, 8.6 Hz) (1 H)	2.41 (dd, 8.6, 14.6 Hz), 2.78 (dd, 5.1, 14.6 Hz)	6.04 (q, 7.5 Hz) (1 H)	1.75 (dd, 7,1 Hz) (3 H)					3.75[c] (s) (3 H), 3.65 (s) (3 H)	2.1 (s), 2.0 (s) (12 H)

[a] All glucosides were determined in D_2O, δ = 0.00 ppm relative to DSS, Acetates were determined in $CHCl_3$, δ = 0.00 ppm relative to Me_4Si. [b] AA'BB' spin system. [c] CH_3O at C-11a and C-11c. [d] CH_3O at C-7a. [e] 1'. [f] 1''. [g] 1'''.

methoxyl, two vinyl (3a and 3c H) and two acetalcarbinyl groups (1a and 1c H), and four aromatic protons, but unlike that of Gl 3, which revealed three different doublets for the glucose anomeric protons, Gl 5 showed only two such protons. Again using the sum of the 3a and 3c vinyl and aromatic protons as the standard for integration, the presence of eight acetylmethyls in the Gl5 acetate was determined. These observations coupled with the elemental analysis and the molecular weight determination of 1310 ± 27 for the Gl 5 octaacetate were consistent with structure **9** for Gl 5, a structure differing from that of Gl 3 in possessing no central glucose unit. Again, as in the case of Gl 3, the elemental analysis was determined on the acetate rather than the glucoside since the latter gave a poorly defined hydrate.

Methanolysis of Gl 5 at 80° produced oleoside 7-methyl ester, identified by comparison of its tetraacetate with authentic sample. Also produced was ligstroside[5] (**10**) identified as its pentaacetate by comparison of its properties with those reported.

^{13}C NMR. Assignments of Gl 3, 5, and 6 resonances were made with the assistance of wide-band off-resonance decoupled spectra and the correlation given in Table II in which methyl β-D-glucopyranoside, salidroside, and oleoside 7-methyl ester serve as the basic models for oleoside aglucon, tyrosol, and glucose units within the three Oleaceae metabolites. Chemical shift deviations as a result of ester or ether linkages could be assessed from the literature and ancillary model studies reported below.

The assignments for methyl β-D-glucopyranoside were taken as reported.[6] Small deviations from the reported chemical shift values were observed but in no case did they exceed 0.3 ppm.

Assignments of the glucosidic portion of salidroside and oleoside 7-methyl ester followed from the methyl β-D-glucopyranoside spectrum. The tyrosol portion of salidroside could be assigned using the off-resonance spectra and the chemical shift effects of substituted benzenes[7] and ethers.[8]

The assignments for the remaining 12 carbons of oleoside 7-methyl ester followed, for the most part, from the off-resonance spectra and known chemical shift relations. Only a few of these assignments require special comment. First, the C-11 carbonyl was placed at higher field than the C-7 carbonyl on the basis that the chemical shift of C-22, the methyl ester carbonyl, in ring D of ajmalicine and reserpinene[9] comes at higher field than the carbonyl of a saturated ester. Secondly, the β-oxy-α,β-unsaturated methyl ester system of ajmalicine and reserpine also served as models for assisting the assignments of C-3 and C-4 in oleoside 7-methyl ester. Finally, it was possible to distinguish conclusively the two acetalcarbinyl carbons, the one (C-1′) in the glucose unit and the other in the oleoside aglucon unit, by single-frequency decoupling. Thus when the C-1′ proton appearing as a doublet at δ 4.92 was irradiated, only the carbon doublet at 100.5 ppm collapsed to a singlet. The result means the 100.5 doublet must be assigned to C-1′ leaving the 95.7 carbon doublet to C-1a by default.

The ^{13}C NMR distinguishes the chain locations of the glucose moieties. The spectrum of Gl 3 exhibits three signals attributable to C-4 in three glucose units. Moreover, one glucose unit is incorporated in a different manner than the other two since signals for a C-1 (102.7 ppm), C-5 (73.5 ppm), and C-6 (64.1 ppm) lie outside the 99% tolerance interval. Similarly, one of two sets of C-1, C-5, and C-6 signals from Gl 6 lies outside the 99% tolerance interval. In these two cases, the deshielding of C-6 and shielding of C-5 agree precisely with the β and γ effects engendered by esterification of aliphatic alcohols, as ascertained from reported data.[10] The variance of C-1 in one glucose unit of Gl 3 and 6 puts these chemical shifts in line with the anomeric carbon in salidroside and methyl β-D-glucopyranoside. However, rather than these signals being unusually downfield, it is the chemical shifts of C-1 in terminal glucose units that are unusual as a result of branching of the oleoside aglucon units at C-1a and C-1c which generate shielding γ-gauche effects at the anomeric carbons of the terminal glucose units. An example of this effect is found in comparing the chemical shifts of the anomeric carbons in the glucose units of maltose and sucrose.[11]

In contrast to the spectra of Gl 3 and 6, the spectra of Gl 5 show no variances in the glucose moiety signals, the chemical shift values of C-1, -5, and -6 being within the 99% tolerance intervals, thus indicating that the two glucose units of Gl 5 are both terminal.

The different involvement of the tyrosol unit in the metabolite chains is apparent from the chemical shifts of both the aromatic and aliphatic carbons. Comparing aromatic carbon shifts of Gl 3 and Gl 5 on the one hand with those of Gl 6 and salidroside on the other shows significant differences in the chemical shift of all carbons except C-4b and -8b, the carbons meta to the aromatic oxygen. These shifts are consistent with Gl 6 and salidroside possessing free phenolic tyrosol units but Gl 3 and 5 containing esterified phenolic tyrosol units as exemplified in the case of acetylating **11** to **12.** Chemical shift changes of the various aromatic carbons are: C-3, +5.9; C-4 and C-8, −0.3; C-5 and C-7, +6.0; and C-6, −6.3. The effect of acylating an aliphatic alcohol was noted above in the discussion of variable chemical shifts of C-5 and C-6 in the glucose units. Typical are the effects of acetylating **13** to **12;** C-1 and C-2 are shifted by +1.3 and −4.1 ppm, respectively. Thus the chemical shift changes at carbons one and two bonds removed from a hydroxyl group are in the opposite direction for acylation of phenolic and alcoholic hydroxyl groups.

The chemical shift of C-1b is the same in Gl 3, Gl 6, and salidroside but is upfield by nearly 5.0 ppm in Gl 5, a change which is consistent with C-1b being ester linked in Gl 5 but acetal linked in Gl 3, Gl 6, and salidroside.[12]

As for the oleoside aglucon units, all chemical shifts for each carbon remain constant throughout the entire series except for one C-7 (C-7c) of Gl 3 and Gl 5 which appears slightly shielded relative to the 90% tolerance interval and therefore indicates a different type of ester linkage for one of two C-7 carbonyls in both Gl 3 and Gl 5. A similar difference can be observed in comparing the carbonyl chemical shifts of **11, 12,** and **13.** The carbonyls of **11** and **13** come at 172.4 and 169.6 ppm, respectively, while in **12** they appear at 171.2 and 169.7 ppm. The somewhat shielded position of the phenolic ester carbonyl is ascribed to the greater steric interaction of this carbonyl with the aromatic ring as compared to the C-1 methylene. While there is some variance in the C-7 carbonyl chemical shifts, there is none in the higher field chemical shift near 169 ppm assigned to the C-11 carbonyl. This result is consistent with C-11 being involved only as methyl esters and C-7 in different ester linkages.

Discussion

The literature is replete with examples of iridoid glycosides combined with various derivatives of β-phenylethanol or benzoic and cinnamic acids, the aromatic hydroxylated modifications being prevalent among them.[13] The structures of Gl 3 and 5 represent the first examples demonstrating the involvement of the same secoiridoid unit in a reoccurring fashion in a polyglycosidic chain.

Likely the discovery of these bis(secoiridoids) rests with the method of isolation, with 70% methanol at 0–10°, but earlier secoiridoid isolations, from various Oleaceae, were performed using methanol at elevated temperatures, conditions which, as we have demonstrated, are sufficient to effect methanolysis of ester linkages, especially the phenolic ester linkages. The apparent necessity of low-temperature isolation suggests that careful reexamination of plant species yielding glucosidic iridoids combined with aromatic ring-hydroxylated β-phen-

Table II. Carbon-13 NMR Chemical Shifts (δ)[a] of Secoiridoid Glucosides

Secoiridoid glucoside	Carbons in the moiety																					
	Oleoside aglucon											Tyrosol						Glucose					
	1	3	4	5	6	7	8	9	10	11	OCH_3	1b	2b	3b	4b, 8b	5b, 7b	6b	1	2	3	4	5	6
Gl 3	95.3[b] d[q]	155.1[c] d	108.4 s	30.5 d	40.3[d] t	173.7 s	125.7[e] d	128.9 s	13.2[f] q	169.0 s	52.1[j] q	70.8 t	35.1 t	137.2 s	130.5 d	121.7 d	149.0 s	100.1 d[n]	73.3[g] d	76.6[h] d	70.0[i] d	76.0[h] d	61.0 t
	95.1[b] d[r]	155.0[c] d	108.4 s	30.5 d	40.2[d] t	172.4[k] s	125.2[e] d	128.9 s	13.1[f] q	169.0 s								102.7[k] d[o]	73.1[g] d	76.6[h] d	70.1[i] d	73.5[g,k] d	64.1[k] t
																		100.1 d[p]	73.1[g] d	76.6[h] d	69.8[i] d	76.0[h] d	61.0 t
Gl 5	95.7[b] d[q]	155.6 d	108.9 s	31.2 d	40.9[c]	174.1 s	125.8[d] d	129.7[e] s	13.8[f] q	169.3 s	52.6 q	66.8 t	34.4 t	137.4 s	131.2 d	122.3 d	150.0 s	100.7 d[r]	73.7 d	77.3[g] d	70.4 d	76.7[g] d	61.7 t
	95.5[b] d[r]	155.6 d	108.9 s	31.2 d	40.7[c]	172.5[k] s	125.6 d	129.4[e] s	13.3[f]	169.3 s								100.7 d[p]	73.7 d	77.3[g] d	70.4 d	76.7[g] d	61.7 t
Nüzhenide (Gl 6)	95.7 d	155.6 d	109.0 s	31.1 d	41.0 t	174.4 s	125.7 d	129.4 s	13.6 q	169.8 s	52.6 q	71.8 t	35.3 t	131.1 s	131.1 d	116.3 d	155.1 s	100.5 d[n]	73.8[b] d	76.6[c] d	70.3[d] d	77.2[c] d	61.5 t
																		103.1[k] d[o]	73.6[b] d	76.6[c] d	70.7[d] d	74.1[b,k] d	64.8[k] t
Oleoside methyl ester	95.7 d	155.6 d	109.1 s	31.0 d	40.6 t	175.6 s	125.9 d	129.2 s	13.2 q	170.1 s	52.0 q 53.0 q							100.5 d	73.4 d	77.1[b] d	70.2 d	76.5[b] d	61.4 t
Salidroside												71.8 t	35.3 t	131.4 s	131.2 d	116.3 d	154.9 s	103.1[k] d[o]	73.9 d	76.7 d	70.5 d	76.7 d	61.6 t
Methyl β-D-glucopyranoside																		104.1[k] d[o]	73.9 d	76.7 d	70.5 d	76.7 d	61.6
Tolerance interval						174.4 ±1.7[m]												100.4 ±1.1[l]	73.6 ±1.0[l]	76.8 ±1.0[l]	70.3 ±0.9[l]	76.6 ±1.4[l]	61.4 ±1.0[l]

[a] In parts per million from Me_4Si and measured in D_2O from internal dioxane as the secondary reference. [b-i] Assignments for any one glucoside can be interchanged when the same superscripts appear. [j] Double intensity. [k] Outlying δ values not selected in calculating the averages, standard deviations, and 90 or 99% tolerance interval. [l] A 99% tolerance interval for an observation of the selected chemical shifts. [m] A 90% tolerance interval for an observation of the selected chemical shifts. [n] 1'. [o] 1". [p] 1"'. [q] 1a. [r] 1c.

ylethanol derivatives would give a better indication of the structural involvement of the three basic units.

Finally we note the utility of ^{13}C NMR in assisting the sequence determination. Internal and terminal glucose units, aromatic and aliphatic esters, and free and esterified phenolic units are in each case distinguishable. Based on this study, utilization of ^{13}C NMR in the further investigation of these types of molecules would be particularly advantageous.

Experimental Section

Spectra were determined as follows: ^{13}C of glucosides in D_2O in 12-mm tubes, chemical shifts in parts per million (0.00 Me_4Si) and measured from dioxane as an internal secondary reference on a Varian XL-100-15 operating at 25.16 MHz in the pulsed Fourier transform and absorption modes and controlled by a Varian VFT-100-16L computer, Fourier transformations based on 8192 data points, field frequency lock established on the deuterium resonance of D_2O, between 1.5 and 22K transients obtained for totally decoupled spectra and nearly four times that number for off-resonance decoupled spectra; ^1H NMR of glucosides in D_2O (δ = 0.00 DSS) and glucoside acetates in $CDCl_3$ (δ = 0.00 Me_4Si) in 5-mm tubes and determined on Varian A-60 and XL-100-15 spectrometers, the latter operating in the pulsed Fourier transform mode, symbols br, d, q, s, and t refer to broad, doublet, quartet, singlet, and triplet, respectively; ir in KBr and in solution as indicated; uv on a Cary-16 in solution as indicated. Melting points were determined on a Köfler micro hot stage and a Mel-Temp apparatus and are uncorrected. Optical rotations were determined in solution as indicated on a Perkin-Elmer 141 polarimeter. The elemental analysis was performed by Galbraith Laboratories, Knoxville, Tenn. Unless specified otherwise, thin-layer chromatography (TLC) was carried out routinely on microscope slides uniformly coated with 0.25 mm of the absorbant indicated and employing the solvent indicated.

Gl 3. A previously isolated homogeneous sample[2] possessed the ^1H and ^{13}C NMR summarized in Tables I and II, respectively. Other spectral and chromatographic properties were TLC (Al_2O_3, HF_{254}, *n*-BuOH saturated with H_2O) R_f 0.3; ir (KBr) 3400 (OH), 1735 (C=O), 1704 (C=O), 1630 (C=O), 1070 cm^{-1} (CO); and uv (95% EtOH) λ_{max} 236 nm (ϵ 23 000).

A 3-mg portion of the sample was treated with 5 drops of 4.7% aqueous $FeCl_3$ at 25°. No coloration developed in 1 h but a violet-blue color developed on standing overnight. Under the same conditions the blank $FeCl_3$ solution retained its yellow color. A second 3-mg portion in 5 drops of dilute aqueous NaOH was heated at 80° for 10 min, acidified with dilute aqueous HCl, and basified with aqueous NH_3, and the water was removed at reduced pressure. To the dry residue was added 5 drops of the aqueous 4.7% $FeCl_3$ solution. A violet-blue color was observed immediately.

Methanolysis of Gl 3. A solution of 96 mg of Gl 3 in 30 ml of methanol was heated at 80° under N_2 for 48 h, the time at which TLC (Al_2O_3, *n*-BuOH saturated with H_2O) showed that no Gl 3 remained. The methanol was removed at reduced pressure and the oily residue was eluted from 7 g of silica gel (Woelm, activity IV) first with MeOH–$CHCl_3$ (1:9) in six, 25-ml fractions and then with MeOH–$CHCl_3$ (1:1) in two 30-ml fractions. Fractions 2 and 3 yielded 51 and 4 mg, respectively, of oleoside methyl ester: TLC (Al_2O_3, HF_{254}, *n*-BuOH saturated with H_2O) R_f 0.47; $[\alpha]^{25}D$ −169° (*c* 2.5, 95% EtOH); uv (95% EtOH) λ_{max} 236.5 nm (ϵ 12 000); ^1H NMR, see Table I; ^{13}C NMR, see Table II.

Fractions 6 and 7 yielded 32 and 13 mg, respectively. A 9-mg portion of the latter was applied to five 10 × 25 cm plates coated with 0.5 mm of silica gel GF_{254} which were developed with $CHCl_3$–MeOH (7:3) and thereafter the two bands corresponding to R_f 0.47 and 0.70 were separately removed and washed with MeOH. The R_f 0.47 band yielded 5 mg of nüzhenide: $[\alpha]^{25}D$ −151° (*c* 1.7, MeOH); uv (95% EtOH) 277 nm (ϵ 2200), 226 (13 400); ^1H NMR (D_2O, TSS), see Table I; ^{13}C NMR, see Table II and identical with those from authentic sample[14] and Gl 6.[2] The R_f 0.70 band afforded 1.2 mg of salidroside whose ^1H NMR (see Table I) was identical with that of an authentic sample.

Fraction 6 was eluted from a wet-packed ($CHCl_3$–MeOH, 9:1) column of 5 g of silica gel (Woelm, activity III) with $CHCl_3$–MeOH (9:1) in 50-, 40-, 15-, 5-, and 30-ml fractions, constituting fractions 1–5, respectively, and 80 ml of $CHCl_3$–MeOH (4:1), constituting fraction 6. Fractions 2 and 6 yielded pure salidroside (3 mg) and nüzhenide (19 mg), respectively. Fractions 3 and 4 (3.1 and 1.1 mg, respectively) contained salidroside with a trace (TLC) of nüzhenide and fraction 5 contained a mixture of salidroside and nüzhenide. Thus in total, from 96 mg of Gl 3 were obtained 51 mg of oleoside 7-methyl ester, 7.2 mg of salidroside, and 24 mg of nüzhenide which amounted to the three products oleoside 7-methyl ester, salidroside, and nüzhenide being formed in a mole ratio of 1.35:0.27:0.39.

Gl 3 Undecaacetate. A solution of 36 mg of Gl 3 in 0.5 ml of pyridine and 0.5 ml of acetic anhydride was kept at 25° for 5 h. Evaporation of solvent and unconsumed acetic anhydride at reduced pressure gave a residue which was eluted from 3 g of neutral alumina (activity 2) with chloroform, the first 2 ml of which was discarded and the next 20 ml yielded 53.6 mg of oily residue after vacuum evaporation. Trituration of this residue with 95% EtOH–$CHCl_3$ gave solid Gl 3 undecaacetate: ^1H NMR, see Table I: ir (CCl_4) 5.68, 5.85, and 6.11 μ; uv (95% EtOH) λ_{max} 235 nm (ϵ 27 000). The analytical sample was prepared by evacuation at 56° over P_2O_5 for 15 days. Anal. Calcd for $C_{70}H_{86}O_{38}$: C, 54.75; H, 5.64. Found: C, 54.65; H, 5.75.

Methyl Oleoside Tetraacetate. A solution of 19.4 mg of oleoside 7-methyl ester in 0.5 ml of pyridine and 0.5 ml of acetic anhydride was kept at 25° for 2 h and thereafter the pyridine and unconsumed acetic anhydride were removed at reduced pressure. The residue, 27.8 mg, was eluted from 6 g of silica gel (Woelm, activity II) first with 20 ml of $CHCl_3$, giving fraction 1, and then with 20 ml of $CHCl_3$–MeOH (95:5), giving fraction 2, and from which was obtained 27 mg of methyl oleoside tetraacetate: TLC [SiO_2, GF_{254}, $CHCl_3$–MeOH (9:1)] R_f 0.9; $[\alpha]^{25}D$ −153° (*c* 1.0, $CHCl_3$); ir (CCl_4) 5.68, 5.82, and 6.10 μ; uv (95% EtOH) λ_{max} 236 nm (ϵ 11 600); ^1H NMR, see Table I.

Gl 5. The following procedure is typical of those employed to isolate Gl 5 from mixtures containing the latter and Gl 3 or Gl 6 (nüzhenide).

A 50-mg sample, previously isolated[2] from *F. americana*, containing Gl 5 and Gl 6 was eluted from 8 g of silica gel (Woelm, activity III) with $CHCl_3$–MeOH (4:1). Two 25-ml, a 30-ml, and a 70-ml fraction were taken in that order. From fraction 4 was isolated 34 mg of pure nüzhenide. Fraction 3 yielded 4 mg of a mixture of Gl 5 and nüzhenide and fraction 2 yielded 8 mg of Gl 5: TLC [SiO_2, GF_{254}, $CHCl_3$–MeOH (7:3)] R_f 0.61; $[\alpha]^{25}D$ −185° (*c* 3.4, MeOH); uv (95% EtOH) λ_{max} 236 nm (ϵ 20 000); ir (KBr) 3400 (OH), 1720 (C=O) 1700 (C=O), 1630 (C=O), 1070 (CO); ^1H NMR, see Table I; ^{13}C NMR, see Table II.

Gl 5 Octaacetate. A solution of 34 mg of Gl 5 in 0.5 ml of pyridine and 0.5 ml of acetic anhydride was kept at 25° for 3 h, the time at which TLC showed that all Gl 5 was consumed. The pyridine and unconsumed acetic anhydride were removed at reduced pressure and the resulting residue was eluted from 4 g of silica gel (activity II) first with 10 ml of $CHCl_3$ and then with 15 ml of 5% MeOH in $CHCl_3$. From the latter fraction was obtained 44 mg of Gl 5 octaacetate: TLC [SiO_2, GF_{254}, $CHCl_3$–MeOH (95:5)] R_f 0.95; $[\alpha]^{25}D$ −136° (*c* 1.1, $CHCl_3$); ir (CCl_4) 5.67, 5.83, 6.10, and 6.65 μ; uv (95% EtOH) λ_{max} 236 nm (ϵ 24 000); ^1H NMR, see Table I. Anal. Calcd for $C_{58}H_{70}O_{16}$: C, 55.85; H, 5.66. Found: C, 55.76; H, 5.82.

Methanolysis of Gl 5. A solution of 8 mg of Gl 5 in 1.5 ml of MeOH was heated at 80° for 11 days at which time TLC (SiO_2, GF_{254}, *n*-BuOH saturated with H_2O) showed a trace of Gl 5 (R_f 0.51) and spots corresponding to R_f 0.59 and 0.73. Removal of MeOH at reduced pressure and elution of the resulting residue from 3 g of silica gel (Woelm, activity III) first with 10 ml, then with 24 0.5-ml fractions, and finally with 100 ml of $CHCl_3$–MeOH (9:1) resulted in partial separation of the methanolysis products. Fractions 2–7 yielded 1.8 mg of oleoside 7-methyl ester, fractions 8–13 yielded 1.7 mg of a mixture of oleoside 7-methyl ester and ligstroside, and the final 100-ml fractions yielded 2 mg of a mixture of Gl 5 and other materials. From fractions 14–24 was obtained 2.5 mg of ligstroside: $[\alpha]^{25}D$ −180° (*c* 0.23, 95% EtOH) [reported[5] −110.7° (*c* 1, EtOH)]; ^1H NMR, see Table I.

Ligstroside Pentaacetate. A 2.3-mg quantity of the ligstroside isolated from methanolysis of Gl 5 was treated overnight at 25° with 0.5 ml of pyridine and 0.5 ml of acetic anhydride. Acetic anhydride and pyridine were removed by vacuum evaporation and the resulting residue, showing only one TLC spot (SiO_2, GF_{254}, twice developed with 1:1 Et_2O–$CHCl_3$, R_f 0.74), was chromatographed on 2 g of silica gel (activity III) by eluting first with 25 ml of $CHCl_3$ and then with 15 ml of CH_3OH–$CHCl_3$ (5:95). The evaporation of the solvent from the latter produced 3.0 mg of ligstroside pentaacetate:[15] $[\alpha]^{25}D$ −127°

(c 0.3, $CHCl_3$); uv (95% EtOH) λ_{max} 232 nm (ϵ 14 000); ir ($CHCl_3$, 3 mg/0.18 ml) 5.70 (s), 5.76 (sh), 6.12 (m), 6.64 (m); ^1H NMR ($CDCl_3$, 3 mg/0.3 ml) δ 1.70 (d of d, J = 7 and 1 Hz, 3 H, C-10a H), 2.03 (br s, 12 H, CH_3CO), 2.30 (s, 3 H, CH_3COOAr), 3.74 (s, 3 H, $COOCH_3$), 5.71 (br s, 1 H, C-1a H), 5.99 (q, J = 7 Hz, C-8a H), 6.98, 7.07, 7.18, 7.27 (AA'BB', 2 H, C-4b, -5b, -7b, -8b H), 7.47 (s, 1 H, C-3a H).

Salidroside. Salidroside tetraacetate was prepared from tetra-*O*-acetyl-α-D-glucopyranosyl bromide and tyrosol according to a previously reported procedure.[15] A solution of 370 mg of salidroside tetraacetate in 5.5 ml of 0.027 N NaOMe in MeOH was kept at 25° for 22 h. Thereafter the solution was filtered through a layer of 2 g of silica gel. Evaporation of the solvents at reduced pressure gave a residue which was eluted from 12 g of silica gel (activity II) with $CHCl_3$–MeOH (6:1). Twelve 35-ml fractions were taken. After removal of the solvent at reduced pressure, fractions 2–5 were triturated with small amounts of $CHCl_3$–MeOH (6:1). Thereby fraction 2 yielded 65.1 mg and fractions 3–5 combined gave 88.8 mg of crystalline salidroside: mp 154–162° (lit.[16] 161–163°); ^1H NMR, see Table I; ^{13}C NMR, see Table II.

Acetylation of Tyrosol. A solution of 690 mg of tyrosol in 7 ml of pyridine was treated with 510 mg of acetic anhydride added dropwise slowly at 25°. The progress of the reaction was monitored by TLC [SiO_2, GF_{254}, C_6H_6–Et_2O (9:1)] until in the course of about 2 h three new spots of nearly equal intensity had developed. Thereafter the solution was treated with aqueous saturated $NaHCO_3$ and then extracted with Et_2O. The extract was dried (Na_2SO_4) and concentrated at the rotary evaporator. The residue was chromatographed on 15 g of silica gel (activity II) by eluting with C_6H_6–Et_2O (9:1) in 35-ml fractions. Fraction 2 contained 182 mg of 2-(4-acetoxyphenyl)ethyl acetate: TLC [C_6H_6–Et_2O (9:1)] R_f 0.8 [visualization by uv irradiation, $KMnO_4$ (slow colorization), and 2% 2,3,5-triphenyltetrazolium chloride in 1.0 N NaOH (no colorization)] compared with tyrosol R_f 0.2 [uv irradiation, $KMnO_4$ (immediate colorization), 2% 2,3,5-triphenyltetrazolium chloride–NaOH (yellow)]; ^1H NMR ($CDCl_3$) δ 1.99 (s, 3 H, CH_3CO), 2.23 (s, 3 H, CH_3CO), 2.87 (t, 2 H, CH_2Ar), 4.21 (t, 2 H, CH_2OAc), 6.93 (m, 2 H, AA' of Ar), 7.15 (m, 2 H, BB' of Ar); ^{13}C NMR δ 20.8 (CH_3), 21.0 (CH_3), 34.5 (C-2), 64.8 (C-1), 121.8 (C-5 and -7), 130.0 (C-4 and -8), 135.7 (C-3), 149.8 (C-6), 169.7 (-COOAr), 171.2 (-$COOCH_2$-).

Fraction 4 contained 20 mg of 2-(4-hydroxyphenyl)ethyl acetate: TLC (C_6H_6–Et_2O, 9:1) R_f 0.7 [uv irradiation, $KMnO_4$ (immediate colorization) and 2% 2,3,5-triphenyltetrazolium chloride–NaOH (yellow)]; ^{13}C NMR δ 21.2 (CH_3), 34.3 (C-2), 65.7 (C-1), 115.8 (C-5 and -7), 129.8 (C-3), 130.3 (C-4 and -8), 155.1 (C-6), 172.4 (-$COOCH_2$-).

Fractions 7–12 yielded a total of 376 mg of 2-(4-acetoxyphenyl)ethanol: TLC (C_6H_6–Et_2O, 9:1) R_f 0.35 [uv irradiation, $KMnO_4$ (slow colorization) and 2,3,5-triphenyltetrazolium chloride–NaOH (no coloration)]; ^1H NMR ($CDCl_3$) δ 2.17 (s, 3 H, CH_3CO), 2.70 (t, 2 H, CH_2Ar), 3.66 (t, 2 H, CH_2OH), 6.80 (m, 2 H, AA' of Ar), 7.05 (m, 2 H, BB' of Ar); ^{13}C NMR δ 21.0 (CH_3), 38.6 (C-2), 63.5 (C-1), 121.8 (C-5 and -7), 130.3 (C-4 and -8), 136.7 (C-3), 149.2 (C-6), 169.6 (-COOAr); MS M^+ m/e 180.

Molecular Weight Determinations by Vapor-Phase Osmometry. A calibration curve was obtained by plotting the instrumentally observed ΔR values against the corresponding molar concentrations for four standard solutions of stachyose acetate (molecular weight 1254) in benzene solution. Values of ΔR then were determined for two concentrations (mg/ml) of Gl 3 undecaacetate in benzene. The observed values of ΔR allowed the molar concentrations to be determined from the calibration curve. In turn, molecular weights of 1580 and 1625 (average 1603 ± 23) then were calculated from the determined molar concentrations and the known concentrations in milligrams per milliliter. Similarly, molecular weight values of 1284 and 1337 (average 1310 ± 27) were obtained from Gl 5 octaacetate in benzene solution.

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