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RESIN GLYCOSIDES. I. ISOLATION AND STRUCTURE ELUCIDATION OF ORIZABIN-I, II, III AND IV, GENUINE RESIN GLYCOSIDES FROM THE ROOT OF IPOMOEA ORIZABENSIS

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Abstract — Four compounds named orizabin I, II, III and IV, which are ether-soluble and correspond to the so-called resin glycoside "jalapin" were isolated, for the first time, from the root of Ipomoea orizabensis (Convolvulaceae). Unlike the presumption advocated by Mannich and Schumann for the ether-insoluble glycoside "convolvulin" from I. purga, all of them were respectively a monomer of hydroxyfatty acid oligoglycoside in which the sugar moiety is partially acylated by organic acids and also combines with the carboxy group of the aglycone to form a macrocyclic ester. Their isolation in a pure state was successfully achieved by a high resolution preparative HPLC in the final step. The structures were determined on the basis of chemical and spectral data and regarded to represent those of genuine ingredients.

The so-called "resin glycosides" are well known as the purgative ingredients, which are characteristic of some crude drugs, such as Pharbitidis Semen, Jalapae Tuber and Mexican Scammony Root originated from the Convolvulaceae plants. Chemical investigations on these resin glycosides were initiated as early as in the middle of the 19th century.[1] When subjected to alkaline hydrolysis, some organic acids (isobutyric, 2-methylbutyric, tiglic acids, etc.) and a hydroxyfatty acid oligoglycoside (glycosidic acid) are provided and the latter is cleaved with acid to yield several kinds of monosaccharide (glucose, rhamnose, fucose, etc.) and a hydroxyfatty acid.

In consideration of its behaviour towards acid and alkali, the ether-insoluble resin glycoside Mayer's "convolvulin"[1c] from I. purga was presumed by Mannich and Schumann[2] in 1938 to be a complex glycoside composed of a number of the repeating unit which is a glycosidic acid partially acylated by some organic acids at the sugar moiety. However, any pure resin glycoside has not so far been isolated and the chemical studies have been confined only to characterization of the component organic acids and glycosidic acids afforded by alkaline hydrolysis of a crude resin glycoside.[3]

The dried sliced root of Ipomoea orizabensis (pellet) Ledanois has been known under various names such as "Mexican Scammony Root", "Light, Fusiform, or Woody Jalap" and "Orizaba Root".[4] The resin obtained from the root is designated Ipomoea resin (Orizabin),[5] of which the ether-soluble portion was called "jalapin (jalappin)" by Mayer.[1c] The hydroxyfatty acid (jalapinolic acid) yielded by alkaline and subsequent acid hydrolyses of Ipomoea resin was considered by Kromer[6] to be 12-hydroxy-13-methylpentadecanoic acid. But the structure was later revised synthetically by Asahina et al.[7] and by Davies et al.[8] to be 11-hydroxypalmitic acid. In 1961, Shellard[5] re-examined the components of this resin and identified seven organic acids (acetic, propionic, isobutyric, tiglic, 2-methylbutyric, n-valeric and isovaleric acids), three sugars (glucose, fucose and rhamnose) together with jalapinolic acid from the ether-soluble portion, and the same organic acids and sugars as above along with ipurolic and convolvulinolic acids from the ether-insoluble

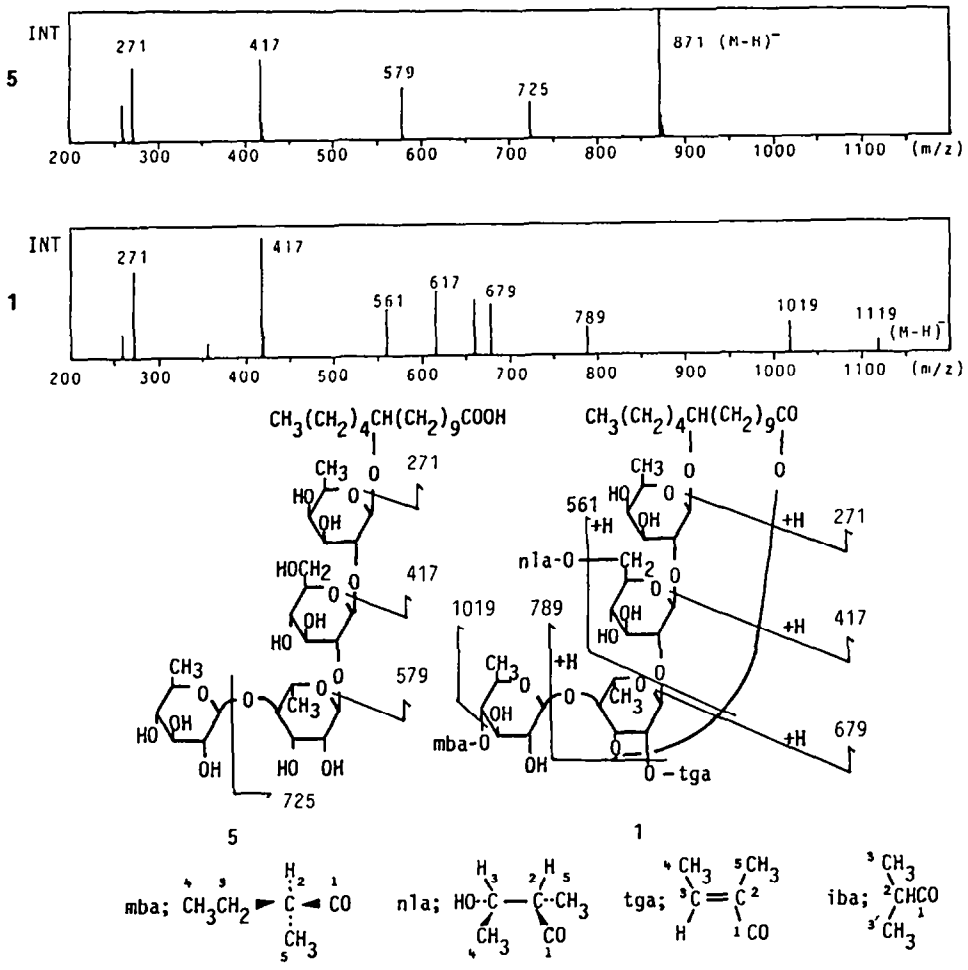


Fig. 1 Negative ion FAB-MS of 1 and 5

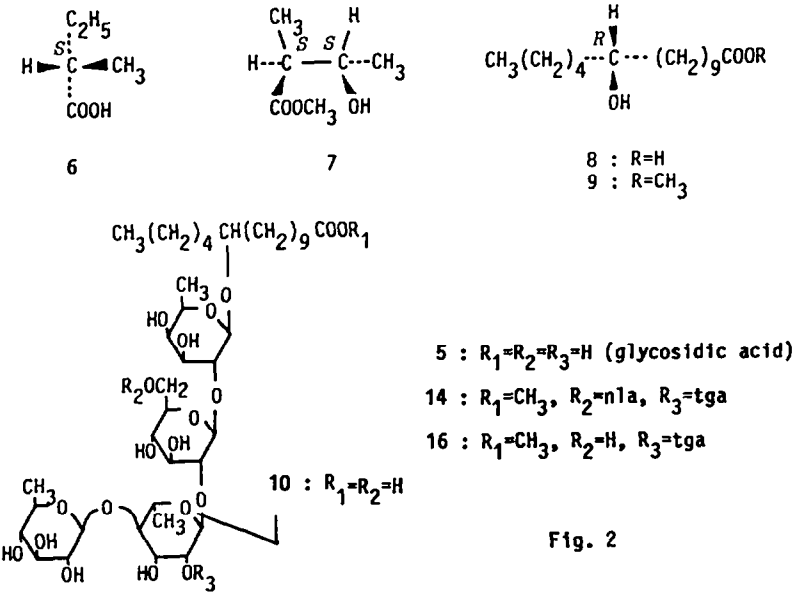


Fig. 2

Table I. ^{1}H-NMR Spectral Data of 1,2,3,4 and thier Derivertives (400 MHz)

		1	2	3	4	5	10	11	12
fuc-	1	4.92(d,8.0)	4.91(d,7.6)	4.91(d,7.6)	4.91(d,7.8)	4.72(d,7.5)	4.83(d,7.5)	4.92(d,7.5)	4.95(d,7.5)
	2	4.60(dd,8.0,9.5)	4.59(dd,7.6,9.2)	4.58(dd,7.6,10.0)	4.58(dd,7.8,9.1)	4.48(dd,7.5,9.0)	4.48(dd,7.5,9.9)	4.60(dd,7.5,9.0)	4.54(dd,7.5,9.5)
	3	4.16(dd,9.5,3.0)	4.16(dd,9.2,3.0)	4.15(dd,10.0,3.5)	4.16(dd,9.1,3.0)	4.29(9.0,3.0)	4.19(dd,9.9,3.1)	4.17(dd,9.0,3.0)	4.10(dd,9.5,3.0)
	4	3.94(d,3.0)	3.93(d,3.0)	3.93(d,3.5)	3.98(d,3.0)	3.83(d,3.0)	4.04(dd,3.1,0.6)	3.96(d,3.0)	3.93(d,3.0)
	5	3.80(q,6.0)	3.79(q,6.4)	3.79(q,6.5)	3.81(q,6.4)	3.69(q,6.0)	3.78(dq,0.6,6.5)	3.87(q,6.5)	3.78(dq,0.6,6.5)
	6	1.52(d,6.0)	1.51(d,6.4)	1.51(d,6.5)	1.51(d,6.4)	1.40(d,6.0)	1.15(d,6.5)	1.52(d,6.5)	1.51(d,6.5)
glc-	1	5.84(d,7.0)	5.81(d,7.3)	5.81(d,7.2)	5.81(d,7.1)	5.62(d,7.0)	5.18(d,7.5)	5.83(d,7.2)	5.77(d,7.2)
	2	4.22(dd,7.0,8.0)	4.22(dd,7.3,10.0)	4.20(dd,7.2,9.0)	4.20(dd,7.1,9.0)	4.10(dd,7.0,9.0)	4.09(dd,7.5,8.5)	4.20(dd,7.2,9.0)	4.29(dd,7.2,9.0)
	3	4.24(dd,8.0,8.0)	4.23(dd,10.0,10.0)	4.22(dd,9.0,9.0)	4.23(dd,9.0,9.0)	4.14(dd,9.0,9.0)	4.16(dd,8.5,8.5)	4.24(dd,9.0,9.0)	4.32(dd,9.0,9.0)
	4	3.97(dd,8.0,8.0)	3.96(dd,10.0,10.0)	3.96(dd,9.0,9.0)	3.96(dd,9.0,9.0)	3.99(dd,9.0,9.0)	4.23(dd,8.5,8.5)	3.90(dd,9.0,9.0)	5.51(dd,9.0,9.0)
	5	3.80(*)	3.80(*)	3.80(*)	3.80(*)	3.60(ddd,3.0,5.0,9.0)	3.84(ddd,3.0,5.0,8.5)	3.87(ddd,3.0,5.0,9.0)	3.75(ddd,3.0,5.0,9.0)
	6	4.60(dd,5.0,11.0)	4.60(dd,5.0,11.5)	4.58(dd,5.5,11.5)	4.59(dd,5.0,11.5)	4.13(dd,5.0,11.5)	4.37(dd,5.0,11.5)	4.60(dd,5.0,11.5)	4.11(dd,5.0,12.0)
		4.87(dd,3.0,11.0)	4.87(dd,3.0,11.5)	4.80(dd,3.0,11.5)	4.87(dd,3.0,11.5)	4.20(dd,3.0,11.5)	4.48(dd,3.0,11.5)	4.87(dd,3.0,11.5)	4.24(dd,3.0,12.0)
rha-	1	6.40(d,1.7)	6.38(d,1.7)	6.38(d,1.5)	6.38(d,1.6)	6.21(d,1.2)		6.39(d,1.5)	6.47(d,1.5)
	2	6.25(dd,1.7,3.0)	6.24(dd,1.7,3.0)	6.23(dd,1.5,3.2)	6.20(dd,1.6,3.0)	4.72(dd,1.2,3.0)		6.22(dd,1.5,3.1)	6.34(dd,1.5,3.5)
	3	6.50(dd,3.0,10.0)	6.48(dd,3.0,10.0)	6.48(dd,3.2,9.9)	6.43(dd,3.0,10.0)	4.81(dd,3.0,9.0)		6.44(dd,3.1,10.0)	6.37(dd,3.5,10.0)
	4	4.40(dd,10.0,10.0)	4.39(dd,10.0,10.0)	4.39(dd,9.9,9.9)	4.43(dd,10.0,10.0)	4.34(dd,9.0,9.0)		4.44(dd,10.0,10.0)	4.39(dd,10.0,10.0)
	5	4.86(dq,10.0,6.0)	4.83(dq,10.0,6.1)	4.82(dq,9.9,6.5)	4.87(dq,10.0,6.3)	5.00(dq,9.0,6.5)		4.88(dq,10.0,6.5)	4.78(dq,10.0,6.5)
	6	1.61(d,6.0)	1.60(d,6.1)	1.60(d,6.5)	1.64(d,6.3)	1.88(d,6.5)		1.64(d,6.5)	1.64(d,6.5)
qui-	1	5.04(d,8.0)	5.02(d,7.6)	5.02(d,7.8)	5.07(d,7.8)	5.11(d,7.2)		5.07(d,7.5)	5.03(d,7.8)
	2	3.86(dd,8.0,9.0)	3.85(dd,7.6,9.5)	3.86(dd,7.8,9.3)	4.22(dd,7.8,9.2)	3.96(dd,7.2,9.0)		3.90(dd,7.5,9.0)	3.84(dd,7.8,9.5)
	3	4.09(dd,9.0,9.0)	4.08(dd,9.5,9.5)	4.10(dd,9.3,9.3)	5.67(dd,9.2,9.2)	4.04(dd,9.0,9.0)		5.70(dd,9.0,9.0)	4.19(dd,9.5,9.5)
	4	5.25(dd,9.0,9.0)	5.22(dd,9.5,9.5)	5.28(dd,9.3,9.3)	3.71(dd,9.2,9.2)	3.57(dd,9.0,9.0)		3.68(dd,9.0,9.0)	5.25(dd,9.5,9.5)
	5	3.67(dq,9.0,6.0)	3.66(dq,9.5,6.5)	3.72(dq,9.3,6.2)	3.72(dq,9.2,6.2)	3.61(dq,9.0,6.0)		3.81(dq,9.0,6.5)	3.67(dq,9.5,6.2)
	6	1.33(d,6.0)	1.30(d,6.5)	1.60(d,6.2)	1.63(d,5.5)	1.52(d,6.0)		1.53(d,6.5)	1.33(d,6.2)
jla-	2	2.66(ddd,3.0,9.0,16.0)	2.65(ddd,3.0,9.0,16.0)	2.65(ddd,3.0,9.0,16.0)	2.48(ddd,3.0,9.0,16.0)	2.48(t,7.0)	2.52(t,7.0)	2.48(ddd,3.0,9.0,16.0)	2.64(ddd,3.0,9.0,16.0)
		2.78(ddd,3.0,9.0,16.0)	2.75(ddd,3.0,9.0,16.0)	2.76(ddd,3.0,9.0,16.0)	2.60(ddd,3.0,9.0,16.0)			2.60(ddd,3.0,9.0,16.0)	2.75(ddd,3.0,9.0,16.0)
	11	3.95(br s)	3.95(br s)	3.96(br s)	3.95(br s)			3.90(br s)	3.98(br s)
	16	0.86(t,7.0)	0.86(t,7.0)	0.86(t,7.0)	0.86(t,7.0)			0.86(t,7.0)	0.84(t,7.0)
tga-	3	7.15(dq,1.0,7.0)	7.14(dq,1.2,7.0)	7.13(dq,1.5,7.0)	7.13(dq,1.5,7.0)			7.13(dq,1.5,7.0)	7.14(dq,1.5,7.0)
	4	1.54(dd,7.0,1.0)	1.28(d,7.0)	1.51(d,7.0)	1.53(d,7.0)			1.54(d,7.0)	1.53(d,7.0)
	5	1.91(d,1.0)	1.91(d,1.2)	1.90(d,1.5)	1.89(d,1.5)			1.88(d,1.5)	1.92(d,1.5)
nla-	2	2.83(dq,7.0,7.0)	2.81(dq,7.0,7.0)	2.81(dq,7.0,7.0)	2.81(dq,7.0,7.0)			2.82(dq,7.0,7.0)	2.75(dq,7.0,7.0)
	3	4.37(dq,7.0,7.0)	4.37(dq,7.0,7.0)	4.36(dq,7.0,7.0)	4.37(dq,7.0,7.0)			4.38(dq,7.0,7.0)	4.36(dq,7.0,7.0)
	4	1.38(d,6.3)	1.37(d,6.3)	1.37(d,6.4)	1.37(d,6.4)			1.37(d,6.4)	1.34(d,6.4)
	5	1.27(d,7.1)	1.28(d,7.0)	1.28(d,7.1)	1.27(d,7.1)			1.27(d,7.1)	1.22(d,7.1)
nla'-	2			2.87(dq,7.0,7.0)					
	3			4.36(dq,7.0,7.0)					
	4			1.43(d,6.4)					
	5			1.33(d,7.0)					
mba-	2	2.49(tq,7.0,7.0)						2.52(tq,7.0,7.0)	2.50(tq,7.0,7.0)
	3	1.50(*),1.89(*)						1.50(*),1.83(*)	1.58(*),1.89(*)
	4	0.95(d,7.0)						0.98(d,7.0)	0.96(t,7.0)
	5	1.20(t,7.0)						1.18(t,7.0)	1.18(d,7.0)
iba-	2		2.63(sept,7.0)		2.65(sept,7.0)				
	3		1.19(d,7.0)		1.17(d,7.0)				
	3'		1.20(d,7.0)		1.22(d,7.0)				

0.02M solution in pyridine-d_5; δ in ppm from TMS (coupling constants, J are in paretheses); fuc, fucose; glc, glucose; rha, rhamnose; qui, quinovose; jla, jalapinolic acid; tga, tiglic acid; nla, nilic acid; mba, 2-methyl-butyric acid; iba, isobutyric acid. *; Signals are overlapping.

Table I. (continued)

	13	14	15	16	17	18	19
fuc- 1	4.92(d,7.6)	4.75(d,7.0)	4.92(d,7.5)	4.79(d,7.5)	4.96(d,7.5)	4.92(d,7.5)	4.96(d,7.5)
2	4.61(dd,7.6,10.0)	4.57(dd,7.0,9.5)	4.59(dd,7.5,9.5)	4.58(dd,7.5,9.5)	4.55(dd,7.5,9.5)	4.60(dd,7.5,9.5)	4.54(dd,7.5,9.5)
3	4.16(dd,10.0,3.5)	4.35(dd,9.5,3.5)	4.14(dd,9.5,3.5)	4.38(dd,9.5,3.0)	4.12(dd,9.5,3.0)	4.15(dd,9.5,3.0)	4.12(dd,9.5,3.0)
4	3.94(d,3.5)	3.82(dd,3.5,0.6)	3.93(dd,3.5,0.6)	3.85(d,3.0)	3.93(dd,3.0,0.6)	3.95(d,3.0)	3.93(dd,3.0,0.6)
5	3.80(q,6.5)	3.72(dq,0.6,6.5)	3.78(dq,0.6,6.5)	3.75(q,6.5)	3.78(dq,0.6,6.5)	3.80(q,6.5)	3.78(dq,0.6,6.5)
6	1.51(d,6.5)	1.43(d,6.5)	1.51(d,6.5)	1.44(d,6.5)	1.52(d,6.5)	1.48(d,6.5)	1.51(d,6.5)
glc- 1	5.83(d,7.2)	5.73(d,7.3)	5.81(d,7.0)	5.75(d,7.5)	5.77(d,7.2)	5.83(d,7.2)	5.77(d,7.2)
2	4.22(dd,7.2,9.0)	4.20(dd,7.3,9.0)	4.25(dd,7.0,9.0)	4.19(dd,7.5,9.0)	4.30(dd,7.2,7.2)	4.20(dd,7.2,9.0)	4.29(dd,7.2,9.0)
3	4.23(dd,9.0,9.0)	4.13(dd,9.0,9.0)	4.27(dd,9.0,9.0)	4.25(dd,9.0,9.0)	4.32(dd,7.2,9.0)	4.23(dd,9.0,9.0)	4.32(dd,9.0,9.0)
4	3.96(dd,9.0,9.0)	4.01(dd,9.0,9.0)	4.06(dd,9.0,9.0)	4.10(dd,9.0,9.0)	5.52(dd,9.0,9.0)	3.96(dd,9.0,9.0)	5.52(dd,9.0,9.0)
5	3.80(*)	3.70(*)	3.65(ddd,3.0,5.0,9.0)	3.63(ddd,3.5,5.0,9.0)	3.75(ddd,3.0,5.0,9.0)	3.80(ddd,3.0,5.0,9.0)	3.78(ddd,3.0,5.0,9.0)
6	4.60(dd,5.0,11.5)	4.68(dd,5.5,11.5)	4.16(dd,5.0,11.0)	4.11(dd,5.0,11.5)	4.15(dd,5.0,11.5)	4.60(dd,5.0,11.5)	4.10(dd,5.0,11.5)
	4.86(dd,3.0,11.5)	4.77(dd,3.0,11.5)	4.23(dd,3.0,11.0)	4.18(dd,3.5,11.5)	4.24(dd,3.0,11.5)	4.86(dd,3.0,11.5)	4.23(dd,3.0,11.5)
rha- 1	6.39(d,1.6)	6.23(d,1.2)	6.49(d,1.5)	6.32(d,1.5)	6.47(d,1.5)	6.38(d,1.1)	6.47(d,1.5)
2	6.25(dd,1.6,3.1)	6.13(dd,1.2,3.0)	6.35(dd,1.5,3.5)	6.14(dd,1.5,3.0)	6.34(dd,1.5,3.5)	6.22(dd,1.1,3.1)	6.34(dd,1.5,3.4)
3	6.51(dd,3.1,10.0)	5.06(dd,3.0,9.5)	6.50(dd,3.5,10.0)	5.11(dd,3.0,9.5)	6.37(dd,3.5,10.0)	6.45(dd,3.1,10.0)	6.37(dd,3.4,10.0)
4	4.44(dd,10.0,10.0)	4.38(dd,9.5,9.5)	4.45(dd,10.0,10.0)	4.39(dd,9.5,9.5)	4.39(dd,10.0,10.0)	4.43(dd,10.0,10.0)	4.38(dd,10.0,10.0)
5	4.86(dq,10.0,6.5)	5.18(dq,9.5,6.0)	4.88(dq,10.0,6.0)	5.15(dq,9.5,6.0)	4.78(dq,10.0,6.5)	4.88(dq,10.0,6.5)	4.78(dq,10.0,6.5)
6	1.66(d,6.5)	2.05(d,6.0)	1.67(d,6.0)	2.06(d,6.0)	1.64(d,6.5)	1.64(d,6.5)	1.63(d,6.5)
qui- 1	5.07(d,8.0)	5.35(d,7.5)	5.07(d,7.5)	5.37(d,7.5)	5.03(d,7.8)	5.10(d,7.5)	5.02(d,7.8)
2	3.87(dd,8.0,8.5)	4.06(dd,7.5,7.5)	3.88(dd,7.5,8.5)	4.09(dd,7.5,9.5)	3.83(dd,7.8,9.5)	4.18(dd,7.5,9.0)	3.86(dd,7.8,9.5)
3	4.07(dd,8.5,8.5)	4.07(dd,7.5,9.0)	4.06(dd,8.5,8.5)	4.15(dd,9.5,9.5)	4.15(dd,9.5,9.5)	5.72(dd,9.0,9.0)	4.20(dd,9.5,9.5)
4	3.70(dd,8.5,8.5)	3.69(dd,9.0,9.0)	3.68(dd,8.5,8.5)	3.66(dd,9.5,9.5)	5.24(dd,9.5,9.5)	3.72(dd,9.0,9.0)	5.29(dd,9.5,9.5)
5	3.71(dq,8.5,6.0)	3.77(dq,9.0,6.0)	3.68(dq,8.5,6.0)	3.77(dq,9.5,6.0)	3.65(dq,9.5,6.2)	3.68(dq,9.0,6.5)	3.70(dq,9.5,6.5)
6	1.57(d,6.0)	1.62(d,6.0)	1.57(d,6.0)	1.62(d,6.0)	1.32(d,6.2)	1.53(d,6.5)	1.43(d,6.5)
jla- 2	2.60(ddd,3.0,9.0,16.0)	2.33(2H,t,7.0)	2.62(ddd,2.0,9.0,15.0)	2.32(t,7.5)	2.65(ddd,3.0,9.0,16.0)	2.48(ddd,3.0,9.0,16.0)	2.63(ddd,3.0,9.0,16.0)
	2.72(ddd,3.0,9.0,16.0)		2.75(ddd,3.0,8.0,15.0)	2.32(t,7.5)	2.77(ddd,3.0,9.0,16.0)	2.60(ddd,3.0,9.0,16.0)	2.74(ddd,3.0,9.0,16.0)
11	3.97(br s)	3.89(br s)	3.99(br s)	3.90(br s)	3.98(br s)	3.95(br s)	3.98(br s)
16	0.86(t,7.0)	0.86(br s)	0.84(t,7.0)	0.86(t,7.5)	0.84(t,7.0)	0.86(t,7.0)	0.84(t,7.0)
tga- 3	7.13(dq,1.5,7.0)	7.12(dq,1.5,7.0)	7.14(dq,1.5,7.0)	7.11(dq,1.5,7.0)	7.15(dq,1.5,7.0)	7.12(dq,1.5,7.0)	7.14(dq,1.5,7.0)
4	1.51(d,7.0)	1.54(d,7.0)	1.53(d,7.0)	1.52(d,7.0)	1.53(d,7.0)	1.54(d,7.0)	1.53(d,7.0)
5	1.89(d,1.5)	1.83(d,1.5)	1.90(d,1.5)	1.82(d,1.5)	1.92(d,1.5)	1.89(d,1.5)	1.92(d,1.5)
nla- 2	2.82(dq,7.0,7.0)	2.82(dq,7.0,7.0)			2.77(dq,7.0,7.0)	2.82(dq,7.0,7.0)	2.75(dq,7.0,7.0)
3	4.38(dq,7.0,7.0)	4.36(dq,7.0,7.0)			4.36(dq,7.0,7.0)	4.36(dq,7.0,7.0)	4.37(dq,7.0,7.0)
4	1.37(d,6.4)	1.38(d,6.5)			1.30(d,6.4)	1.37(d,6.5)	1.30(d,6.5)
5	1.28(d,7.1)	1.28(d,7.0)			1.22(d,7.0)	1.26(d,7.0)	1.17(d,7.0)
nla'-2						2.88(dq,7.0,7.0)	2.89(dq,7.0,7.0)
3						4.22(dq,7.0,7.0)	4.24(dq,7.0,7.0)
4						1.41(d,6.5)	1.32(d,6.5)
5						1.27(d,7.0)	1.26(d,7.0)
mba- 2							
3							
4							
5							
iba- 2					2.65(sept,7.0)		
3					1.18(d,7.0)		
3'					1.20(d,7.0)		
$COOCH_3$		3.64(s)		3.63(s)			

portion. But, the parent glycosides were not isolated.

We have recently succeeded, for the first time, in isolation of four resin glycosides named orizabin I (**1**), II (**2**), III (**3**) and IV (**4**) from the ether-soluble portion of Ipomoea resin. The present paper concerns the details of their isolation and structure elucidation.

The ether-soluble portion exhibited several green spots on Si-gel TLC by spraying 5 % H_2SO_4 in methanol followed by heating on a hot stage and, simultaneously, afforded characteristic odors arising from the component organic acids. It was chromatographed on Si-gel, Bio Beads SX-2 and Lobar Si-gel columns to afford a crude glycoside (26.9 %). It was further separated by a medium pressure preparative HPLC (Si-gel, theoretical plate No.: 10000 - 30000) to give four glycosides, **1**, **2**, **3** and **4**, unexpectedly as colourless needles, in the yields of 4.6, 4.4, 4.1 and 0.4 %, respectively. Their purities were checked by HPLC and the 400 MHz proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Compounds **2**-**3** were considered to be pure but **1** was contaminated by a very small amount of an unknown compound, very likely to be a resin glycoside, but its separation by HPLC was in failure (Fig. 3).

Compounds **1**-**4** were respectively saponified with 5 % KOH to provide a mixture of organic acids together with a glycosidic acid. The organic acids were derivatized to methyl esters and examined by gas chromatography (GC), while a glycosidic acid was examined by ^{1}H-NMR.

Organic acids provided from **1** were tiglic, nilic (3-hydroxy-2-methylbutyric)[9] and 2-methylbutyric acids. Both **2** and **4** gave equally tiglic, nilic and isobutyric acids. Compound **3** furnished tiglic and nilic acids. The glycosidic acids (**5**) yielded from **1**, **2**, **3** and **4** were proved to be identical by ^{1}H-NMR.

Because of shortage of substances for determination of the stereo-structure of the optically active organic acids and the glycosidic acid (**5**), the crude glycoside was saponified and the products were used in the following experiments.

Repeated distillation of the organic acid mixture furnished pure 2-methylbutyric acid (**6**), bp 70°/20 mmHg, $[\alpha]_D$ +17.0°. According to Hirota *et al.*,[10] the chirality at C_2 of (+)-2-methylbutyric acid is *S*.

Treatment of the residue of the above distillation with diazomethane followed by redistillation gave methyl nilate (**7**), bp 85°/18 mmHg, $[\alpha]_D$ +27.8°, of which the stereochemistry at C_2 and C_3 was defined to be 2*S* and 3*S* by referring to the physical data reported by Tai *et al.*[11]

Acidic hydrolysis of the glycosidic acid fraction gave an aglycone (**8**), mp 63°, EI-MS *m/z*: 272 $(M)^+$ together with a sugar mixture. Treatment of **8** with diazomethane gave a compound **9**, mp 42.5-43.3°, $[\alpha]_D$ +1.2°, which was identified with methyl jalapinolate (methyl 11-hydroxypalmitate).[12] The configuration at C_{11} of **9** was proved, for the first time, to be *R* by Horeau's method.[13] Hence, **8** is defined as 11*R*-hydroxypalmitic acid (jalapinolic acid). Chromatographic separation of the sugar mixture gave D-fucose ($[\alpha]_D$ +74.3°), D-glucose ($[\alpha]_D$ +53.0°), L-rhamnose ($[\alpha]_D$ +8.5°) and D-quinovose ($[\alpha]_D$ +28.6°).

On Si-gel chromatography, the glycosidic acid fraction was separated into two homogeneous compounds, **5** and a minor glycoside (*ratio*, *ca.* 8:1). Compound **5** showed four anomeric carbon signals in the ^{13}C-NMR spectrum and its negative ion fast atom bombardment spectrum (FAB-MS) exhibited the peaks at *m/z* 871 $(M-H)^-$, 725 (871-146 (methyl pentose unit))$^-$, 579 $(725-146)^-$, 417 (579-162(hexose unit))$^-$ and 271 $(417-146)^-$ (jalapinolic acid-H)$^-$ (Fig. 2). Permethylation and methanolysis of **5** liberated methyl 3,4-di-O-methly-fucopyranoside, methyl 3,4,6-tri-O-methyl-glucopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside and methyl 2,3,4-tri-O-methyl-quinovopyranoside together with methyl jalapinolate, indicating that **5** is a linear tetraglycoside of jalapinolic acid having the quinovose unit at terminal. Partial acid hydrolysis of **5** gave a diglycoside (**10**), which showed the anomeric proton signals at δ 4.83 (d, *J*=7.5 Hz) and 5.18 (d, *J*=7.5 Hz) in the ^{1}H-NMR spectrum. Compound **10** exhibited a peak at *m/z* 579 $(M-H)^-$ together with fragment peaks at *m/z* 417 $(579-162)^-$ and 271 $(417-146)^-$ (jalapinolic acid-H)$^-$ in the negative ion FAB-MS, and, on complete hydrolysis, gave fucose, glucose and jalapinolic acid.

The *J* values of the anomeric proton and methine proton signals due to sugar moiety in the ^{1}H-NMR of **5** showed that the glycosidic linkages of fucose, quinovose, glucose and rhamnose are β, β,

β and α, respectively.

Consequently, 5 is 11R-hydroxypalmitic acid (jalapinolic acid) 11-O-β-D-quinovopyranosyl-(1-4)-α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranosyl-(1-2)-β-D-fucopyranoside (Fig. 2).

Orizabin I (1), in the negative ion FAB-MS (Fig. 1), exhibited a peak at m/z 1119 but no peak in the higher region up to m/z 3000. Accordingly, the molecular weight of 1 was regarded as 1120. If compound 1 consists of one mole glycosidic acid (5) and one mole each of tiglic, nilic and 2-methylbutyric acids which are combined with the hydroxy groups of the sugar moiety of 5, its molecular weight should be 1138. The lack of 18 mass units might be considered to be due to minus H_2O. It suggests that the carboxy group of the aglycone (jalapinolic acid) is intramolecullaly linked with a hydroxy group of the sugar moiety to form a macrocyclic ester ring.

In order to verify the above suggestion, the 400MHz ^{1}H-NMR, particularly the ^{1}H-^{1}H two-dimensional shift correlated NMR (COSY) spectra were taken and a detailed assignment of proton signals was performed. The ^{1}H-NMR spectrum of 1 (Fig. 3) showed four anomeric proton signals, at δ 4.92 (fucose), 5.04 (quinovose), 5.84 (glucose) and 6.40 (rhamnose). In comparison with that of 5, the remarkable downfield shifts of the signals of 6-H_2 (0.47, 0.67 ppm) of glucose, those of 2-H, 3-H (1.53, 1.69 ppm) of rhamnose and 4-H (1.68 ppm) of quinovose are attributable to acylation. Furthermore, two signals at δ 2.66 (1H, ddd) and 2.78 (1H, ddd) due to unequivalent 2-H_2 of jalapinolic acid moiety, were observed instead of the triplet at δ 2.48 (2H, J=7.0) in 5. Based on the above observation, it is evident that 1 is composed of one mole each of tiglic, nilic and 2-methylbutyric acids and glycosidic acid (5) of which the carboxy group is combined with a hydroxy group of the sugar moiety, and that the locations of acyloxy groups are C_6 of glucose, C_2, C_3 of rhamnose and C_4 of quinovose. Specification of the sites of ester linkages of the respective organic acids and jalapinolic acid was, however, in failure even by using nuclear Overhauser effect spectroscopy (NOESY) and partially relaxed fourier transform (PRFT) methods.

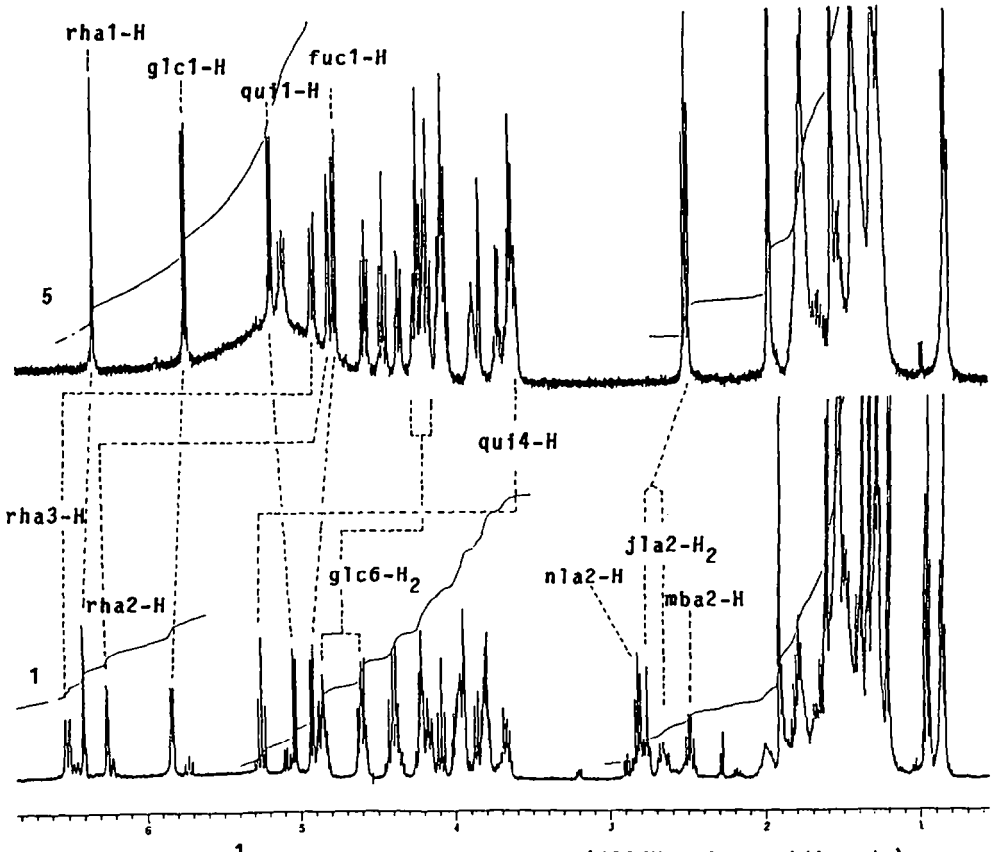


Fig. 3 ^{1}H-NMR Spectra of 1 and 5 (400MHz, in pyridine-d_5)

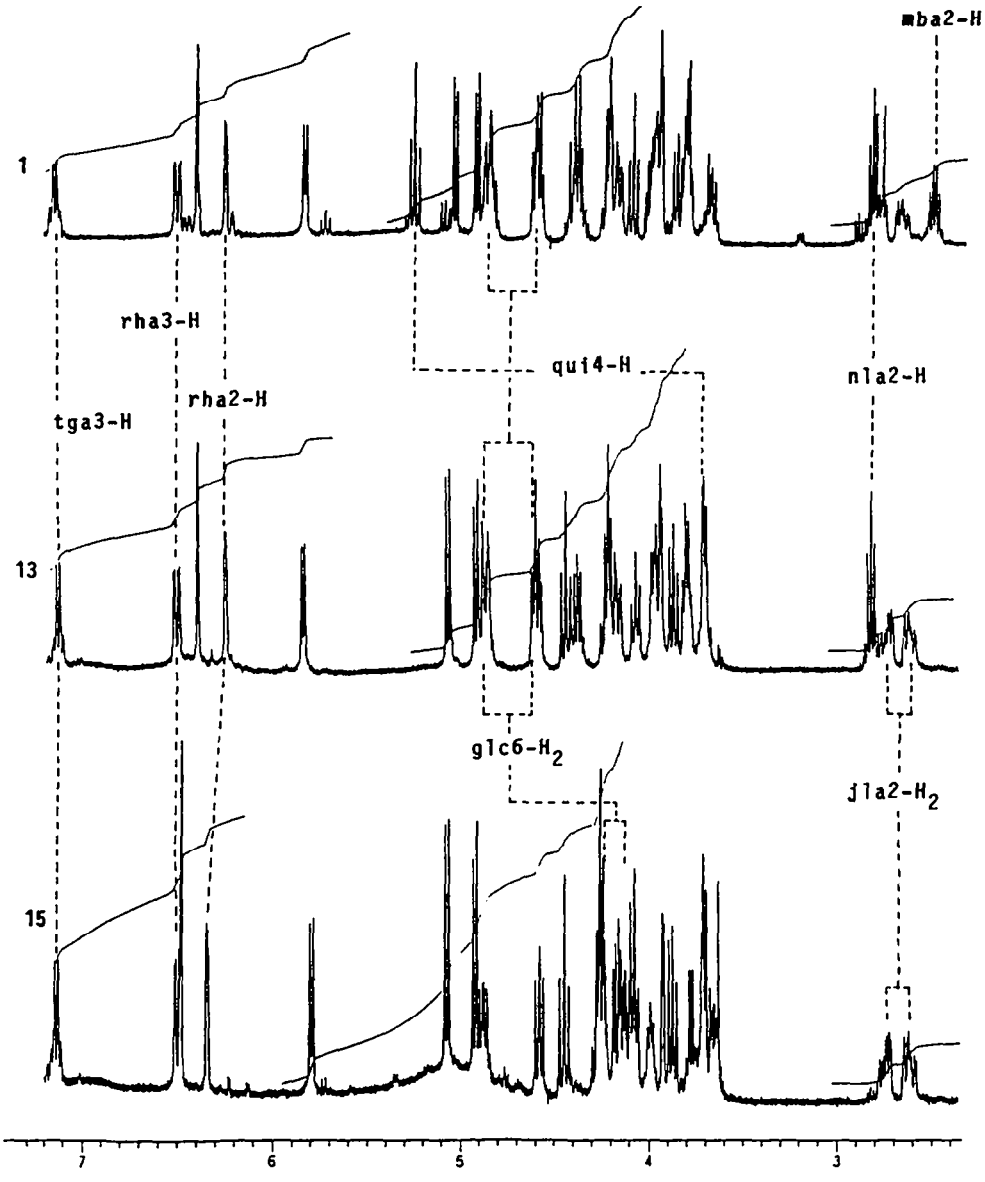


Fig. 4 ^{1}H-NMR Spectra of 1, 13 and 15 (400MHz, in pyridine-d_5)

The negative ion FAB-MS of 1 exhibited, besides the $(M-H)^-$ ion, peaks at m/z 1019, 789, 679, 561 and 417 which were assignable, respectively, to the fragment ions as shown in Fig. 1 suggesting that 2-methylbutyric and nilic acid residues might be located, respectively, at C_4 of the terminal quinovose and at C_6 of the glucose unit. But, the sites of ester linkages of tiglic and jalapinolic acids, both of which are in rhamnose unit, were not discernible.

In order to confirm the above suggestion and to determine the locations of tiglic and jalapinolic acids, partial deacylation and preparative HPLC separation of the products were tried. It is well known[14] that partial deacylation of acylated saccharides under acidic and basic conditions is usually accompanied by acyl-migration to yield a complicated mixture of the products.

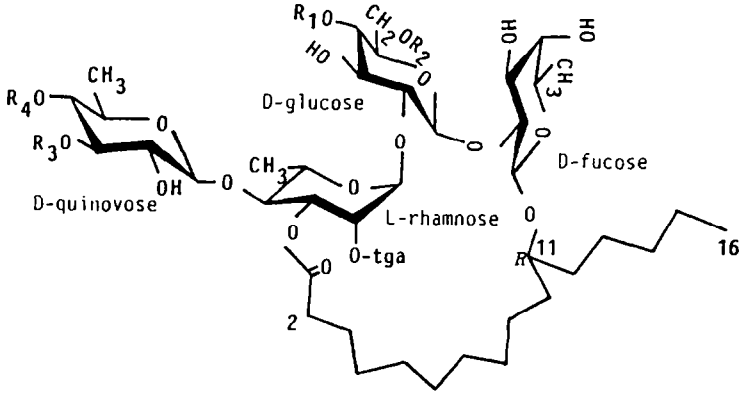
Compound 1 was treated with triethylamine and methanol (1:6) for 30 min at room temperature and the products were separated by HPLC to give three compounds, 11 (2.4 %), 12 (2.2 %) and 13 (9.0 %)

together with recovered **1**. Compound **13** showed a peak at m/z 1035 $(M-H)^-$ in the negative FAB-MS, suggesting that 2-methylbutyric acid was liberated from **1**. The ^{1}H-NMR spectrum of **13** (Fig. 4) revealed, when compared with that of **1**, an upfield shift (1.55 ppm) of the 4-H signal of quinovose and disappearance of the signals due to 2-methylbutyric acid group. Therefore, 2-methylbutyric acid in **1** is considered to be linked with 4-OH of quinovose.

On further deacylation of **13** with 7 % NH_3 in methanol and H_2O (1:1) for 1.5 hr at room temperature, it gave three compounds, **14** (6.7 %), **15** (6.1 %) and **16** (1.2 %). Their negative ion FAB-MS and ^{1}H-NMR spectra were taken and the latters were respectively compared with that of **13**. The MS of **14** showed a peak at m/z 1067 $(M-H)^-$ indicating that the linkage of jalapinolic acid with a hydroxy group of rhamnose was cleaved and that its carboxy group was methylated. The ^{1}H-NMR spectrum of **14** showed a signal at δ 3.64 (3H, s) due to the ester methyl protons, a considerable upfield shift (1.45 ppm) due to 3-H of rhamnose and the equivalent 2-H_2 signals (triplet) due to jalapinolic acid. Thus, the carboxy group of jalapinolic acid should be combined with 3-OH of rhamnose in **1**.

The negative ion FAB-MS of **15** showed a peak at m/z 935 $(M-H)^-$, suggesting that nilic acid was released from **13**. In the ^{1}H-NMR spectrum of **15**, the upfield shifts (0.44, 0.63 ppm) of 6-H_2 of glucose were observed and the signals attributable to the nilic acid moiety disappeared (Fig. 4). Hence, nilic acid in **1** should be attached to 6-OH of glucose.

The ^{1}H-NMR spectrum of **16** (negative ion FAB-MS: $(M-H)^-$, m/z 967) showed a singlet at δ 3.63 (3H), a triplet and the signals ascribable respectively, to those of the ester methyl protons, 2-H_2 of the jalapinolic acid moiety and the tiglic acid residue, together with a considerable upfield shift (1.40 ppm) of the 3-H signal and the acylation shift of 2-H of rhamnose. Therefore, the tiglic acid residue is to be located at 2-OH of rhamnose in **1**.



1 : R_2=nla, R_4=mba, R_1=R_3=H (orizabin I) **2** : R_2=nla, R_4=iba, R_1=R_3=H (orizabin II)

3 : R_2=R_4=nla, R_1=R_3=H (orizabin III) **4** : R_2=nla, R_3=iba, R_1=R_4=H (orizabin IV)

11 : R_1=R_4=H, R_2=nla, R_3=mba **12** : R_1=nla, R_4=mba, R_2=R_3=H

13 : R_1=R_3=R_4=H, R_2=nla **15** : R_1=R_2=R_3=R_4=H

17 : R_1=nla, R_2=R_3=H, R_4=iba **18** : R_1=R_4=H, R_2=R_3=nla

19 : R_1=R_4=nla, R_2=R_3=H

Fig. 5

The compounds **11** and **12**, their negative ion FAB-MS were identical with that of **1**, were characterized as follows. When the ^{1}H-NMR spectrum of **11** was compared with that of **1**, 4-H of quinovose was shifted upfield (1.57 ppm), in contrast to a downfield shift (1.61 ppm) of 3-H of quinovose, while other proton signals assignable to the sugar moiety remain almost unshifted. Therefore, **11**

was regarded as an artefact provided from 1 by migration of 2-methylbutyric acid group from 4- to 3-OH of quinovose. Compound 12 showed, in contrast to 1, a downfield shift (1.54 ppm) of 4-H of glucose and the upfield shifts (0.49, 0.63 ppm) of 6-H_2 of glucose indicating the linkage of nilic acid residue with 4-OH of glucose and not with 6-OH as in 1.

Taking the J values of anomeric and methine proton signals due to sugar moiety into account, the rhamnose unit of 1 is considered to have the $^1C_4$ and other sugar units the $^4C_1$ conformations.

On the basis of all the results described above, the full structure of orizabin I is defined as 11R-hydroxypalmitic acid (jalapinolic acid) 11-O-(4-O-(2S-methylbutyryl))-β-D-quinovopyranosyl-(1-4)-(2-O-tigloyl)-α-L-rhamnopyranosyl-(1-2)-(6-O-(2S-methyl-3S-hydroxybutyryl))-β-D-glucopyranosyl-(1-2)-β-D-fucopyranosyl-1,3(rhamnose)-olide (1 in Fig. 5).

Orizabin II (2) exhibited a negative ion FAB-MS similar to that of 1 except for the $(M-H)^-$ ion peak at m/z 1105. In the ^{1}H-NMR spectrum of 2, the chemical shifts of almost all protons were also similar to those of 1, but, in place of the 2-methylbutyric acid group in 1, the signals due to isobutyric acid appeared. Thus, the structure of 2 is presumed to be analogous to 1 and the 2-methylbutyric acid residue at 4-OH of quinovose in 1 is replaced by isobutyric acid in 2.

On treatment with triethylamine and methanol (1:6), 2 gave three compounds, 4 (2.5 %), 13 (6.5 %) and 17 (2.0 %). The ^{1}H-NMR spectrum of 13 showed, when compared with that of 2, a pronounced upfield shift (1.52 ppm) of 4-H of quinovose and disappearance of the signals due to isobutyric acid. Thus, the presumption was confirmed and orizabin II is assigned the structure 2 in Fig. 5.

The ^{1}H-NMR spectrum of 4, in comparison with that of 2, showed the upfield (1.51 ppm) and downfield (1.59 ppm) shifts, respectively, of 4- and 3-H of quinovose. Therefore, the isobutyric acid group should be linked with 3-OH of quinovose and not with 4-OH as in 2 (Fig. 6). By comparing the ^{1}H-NMR spectra of 2 and 17, 17 was found to be an acyl-migration product, in which the nilic acid residue originally combined with 6-OH in glucose of 2 had migrated to 4-OH of glucose.

Orizabin III (3) exhibited the $(M-H)^-$ peak at m/z 1135 in the negative ion FAB-MS, and gave the ^{1}H-NMR spectrum which was almost same as that of 1, except for the presence of the signals ascribable to two nilic acid residues instead of those due to one 2-methylbutyric acid and one nilic acid residue in 1.

Partial deacylation of 3 in the same way as for 2 gave three compounds, 13 (4.8 %) identical to the partially deacylated product (13) of 1, 18 (2.1 %) and 19 (1.0 %). The ^{1}H-NMR spectrum of 13 showed, when compared with that of 3, an upfield (1.58 ppm) shift of the 4-H of quinovose and disappearance of the signals due to one nilic acid residue. Therefore, one of the two nilic acid groups is located at 4-OH of quinovose, another at 6-OH of glucose and the tiglic acid group is linked with 2-OH of rhamnose. Thus, 3 is assigned the structure shown in Fig. 5.

Compounds 18 and 19 afforded the spectra quite similar to that of 3 in the negative ion FAB-MS indicating that they are isomeric to one another. The structures of 18 and 19 were defined as shown in Fig. 5 by comparison of their ^{1}H-NMR spectra with that of 3 in the same way as for 11 and 12. They are regarded to be the acyl-migration products yielded from 3.

Orizabin IV (4) was identical to the acyl migrated product (4) of 2 (*vide supra*) in the negative ion FAB-MS (m/z: 1105 $(M-H)^-$) and the ^{1}H-NMR spectra.

It might not be excluded that compound 4 could be an artefact yielded from 2, but all the compounds 1-4 were detected in the ether-soluble fraction of the crude drug by HPLC and fairly stable under the conditions of storage and separation at room temperature as shown in Fig. 6. Accordingly, 4 as well as 1-3 could be supposed to be the genuine glycosides which exist originally in the crude drug.

In consideration of their components and structures, four resin glycosides isolated in this study are safely regarded as the so-called resin glycosides and, because of its solubility in ether, to correspond to Mayer's "jalapin".[1c,15] It is noted that they are the monomers having intramolecular macrocyclic ester structure, and that differ from the presumption proposed by Mannich and Schumann for Mayer's "convolvulin", the ether-insoluble resin glycoside of *I. purga*.

An ether-insoluble fraction was obtained in a small amount also from Ipomoea resin, and is under investigation.

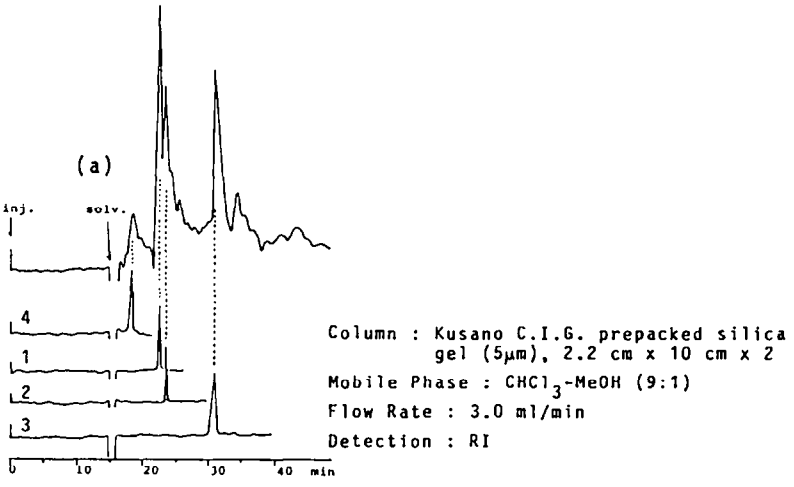


Fig. 6 HPLC of Ether-Soluble Fraction (a) and 1-4 (stored for about one year)

EXPERIMENTAL

All melting points (mp) were determined on a YANACO MP-S3 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM GX-400 spectrometer at 399.65 MHz for ca. 0.02 M solutions at 26° using tetramethylsilane as an internal reference. The abbreviations used are as follows: s, singlet; d, doublet; dd, double-doublet; dq, double-quartet; ddd, double-double-doublet; ddq, double-double-quartet; t, triplet; tq, triple-quartet; sept, septet; br s, broad singlet; m, multiplet. ^{1}H-^{1}H shift-correlated (COSY) spectra were measured by the use of a correlation sequence with 45° mixing pulse and N-type peak selection. Data processing was performed with the standard JEOL software with a 512 x 2048 data point matrix. MS were produced on a JEOL JMS DX-300 spectrometer equipped with a JMA 3500 data system (EI-MS: ionization voltage, 30 eV; accelerating voltage, 3 kV, negative ion FAB-MS: accelerating voltage, 2-3 kV; matrix, triethanolamine; collision gas, Xe). Intensities expressed in % for total ion are in parentheses. Analytical GC was carried out with Shimadzu gas chromatograph GC-8A. IR spectra were taken on a JASCO A-302 spectrometer and optical rotations were measured at room temperature (15-25°) with a JASCO DIP-140 polarimeter. Rotations of monosaccharides were determined 20 h after preparing the solution. TLC was performed on Si-gel precoated Al sheets (Merck, Art 5554) using developing solvents, solv. 1, $CHCl_3$-MeOH (8:2); solv. 2, $CHCl_3$-MeOH-H_2O (6:4:1); solv. 3, n-hexane-AcOEt (4:1), detection with 5 % H_2SO_4-MeOH, and HPTLC plates (Merck, Art 5556) with solv. 4, $CHCl_3$-MeOH-H_2O (6:4:1) and solv. 5, $CHCl_3$-MeOH-H_2O (7:3:0.5) were employed for saccharides. For column chromatography, silica gel 60 (230-400 mesh, Merck), Avicel SF (Funakosi Pharmaceutical Co., Ltd.) and Bio Beads SX-2 (200-400 mesh, Bio Rad Lab.) and Lobar prepacked column (LiChroprep Si60, size C, Merck) were used. Preparative HPLC was conducted on a Kusano C.I.G. prepacked Si-gel column (22 mm i.d. x 30 cm, Kusano Kagakukikai Co.) under pressure 30-50 kg/cm^2.

Isolation of Orizabin I (1), II (2), III (3) and IV (4)

The powdered dry roots (800 g) were percolated successively with n-hexane (1.5 l), chloroform (3 l) and methanol (1.5 l) at room temperature, and each extracts were, respectively, evaporated in vacuo to give an oil (4.3 g), pale yellow powder ("jalapin"[15], 215 g) and dark brown solid (22 g). Chromatography of "jalapin" on Si-gel ($CHCl_3$-MeOH, 10:1) afforded three fractions, F-1 (7.2 g), F-2

(170 g) and F-3 (30 g). Fraction F-2 was rechromatographed over SX-2 (benzene-AcOEt, 1:1) to yield a pale yellow powder (crude resin glycoside, 150 g, 18.8 %) of which separation on Si-gel Lobar column afforded two fractions, fr-1 (118.2 g) and fr-2 (31.8 g). Fr-1 (2.2 g) was subjected to repetitive preparative HPLC ($CHCl_3$-MeOH-H_2O, 9:2:0.1) to afford four resin glycosides, orizabin I (1): colorless needles (n-hexane-acetone (3:1)) (680 mg, 4.6 %), mp 115-121° (dec); $[\alpha]_D$ -5.5° (c 1.3, MeOH); IR (KBr): 3400 (OH), 1720 (C=O) cm^{-1}; negative ion FAB-MS: see Fig. 1; Rf 0.61 (solv. 1); (Found: C, 57.2; H, 8.2. Calc. for $C_{55}H_{92}O_{23}\cdot 2H_2O$: C, 57.1; H, 8.4 %); ^{1}H-NMR: see Fig. 3 and Table I. orizabin II (2): colorless needles (n-hexane-acetone (3:1)) (650 mg, 4.4 %), mp 114-119° (dec); $[\alpha]_D$ -9.7° (c 1.0, MeOH), IR (KBr): 3400 (OH), 1720 (C=O) cm^{-1}; negative ion FAB-MS m/z: 1105 (0.1) $(M-H)^-$, 1005 (0.9), 789 (0.1), 679 (0.5), 661 (0.6), 617 (1.3), 561 (0.5), 417 (2.9), 271 (2.2); Rf 0.61 (solv. 1); (Found: C, 57.6; H, 8.3. Calc. for $C_{54}H_{90}O_{23}\cdot H_2O$: C, 57.6, H, 8.2 %); ^{1}H-NMR: see Table I, orizabin III (3): colorless needles (n-hexane-acetone (3:1)) (610 mg, 4.1%), mp 118-123° (dec), $[\alpha]_D$ -13.3° (c 1.1, MeOH); IR (KBr): 3400 (OH), 1720 (C=O) cm^{-1}; negative ion FAB-MS m/z: 1135 (0.1), $(M-H)^-$, 1035 (0.5), 789 (0.2), 679 (0.5), 661 (0.6), 617 (0.9), 561 (0.6), 417 (2.9), 271 (1.9); Rf 0.58 (solv. 1); (Found: C, 57.3; H, 8.2. Calc. for $C_{55}H_{92}O_{24}\cdot H_2O$: C, 57.2; H, 8.2 %); ^{1}H-NMR: see Table I, orizabin IV (4): colorless needles (n-hexane-acetone (3:1)) (60 mg, 0.4 %), mp 114-119° (dec); $[\alpha]_D$ -5.2° (c 0.9, MeOH); IR (KBr): 3400 (OH), 1720 (C=O) cm^{-1}; negative ion FAB-MS m/z: 1105 (0.1)$(M-H)^-$, 1005 (0.4), 789 (0.1), 679 (0.5), 661 (0.3), 617 (0.6), 561 (0.5), 417 (1.8), 271 (1.4); Rf 0.68 (solv. 1); (Found: C, 57.9; H, 8.2. Calc. for $C_{54}H_{90}O_{23}\cdot H_2O$: C, 57.6; H, 8.2 %); ^{1}H-NMR: see Table I.

Saponification of 1, 2, 3 and 4

Solutions of 1, 2, 3 and 4 (each 12 mg) in 5 % KOH (H_2O-1,4-dioxane (3:1)) (10 ml) were respectively refluxed for 1h. The reaction mixture was made acidic to pH 4.0 with 2 N HCl, and extracted with ether (10 ml). The organic layer was treated with diazomethane and the product was examined by GC (Unisol F-200, 4 mm i.d. x 2 m glass column; column temperature 70°; N_2 2 kg/cm^2). 1: t_R (min) 2.0 (methyl 2-methylbutyrate), 7.4 (methyl tiglate), 24.4 (methyl nilate).[9b] 2: t_R (min) 0.9 (methyl isobutyrate), 7.3 (methyl tiglate), 24.3 (methyl nilate). 3: t_R (min) 7.3 (methyl tiglate), 24.3 (methyl nilate). 4: t_R (min) 0.9 (methyl isobutyrate), 7.3 (methyl tiglate), 24.3 (methyl nilate).

The aqueous phase was extracted with n-BuOH (5 ml) and the organic layer was evaporated in vacuo to give glycosidic acid (5) (each, 5 mg). All the glycosidic acids yielded from 1, 2, 3 and 4 were shown to be identical by ^{1}H-NMR. 5: white powder, mp 120-125°(dec); $[\alpha]_D$ -38.5° (c 2.0, MeOH); IR (KBr): 3400 (OH), 1720 (C=O) cm^{-1}; negative FAB-MS: see Fig. 1; Rf 0.33 (solv. 2); ^{1}H-NMR: see Table I; (Found (in the form of p-bromophenacyl ester): C, 53.9; H, 7.4. Calc. for $C_{48}H_{77}O_{21}Br$: C, 53.9; H, 7.3 %).

Preparation and Identification of 2S-Methylbutyric Acid (6), Methyl 2S,3S-Nilate (7) and glycosidic acid (5) from Crude Resin Glycoside

The crude resin glycoside fraction (25.5 g) was dissolved in 5 % KOH (H_2O-1,4-dioxane, 3:1) (100 ml) and heated at 90° for 3h. The reaction mixture was acidified (pH 4.0) and shaken with ether. The organic layer was dried ($MgSO_4$) and removal of the solvent gave a mixture of organic acid, oil (2.5 g). It (1 g) was subjected fractional distillation to afford 6, colorless liquid (310 mg), bp 70°/20 mmHg; $[\alpha]_D$ +17.0° (c 3.0, MeOH); IR (neat): 2970, 1710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=7.2 Hz, 3-CH_3), 1.18 (3H, d, J=7.0 Hz, 2-CH_3), 1.51 (1H, ddq, J=7.0, 7.0, 7.2 Hz, 3-H), 1.72 (1H, ddq, J=7.0, 7.0, 7.2 Hz, 3-H), 2.40 (1H, tq, J=7.0, 7.0 Hz, 2-H).

The residue (205 mg) of the above distillation was methylated with diazomethane and distilled to give 7, colorless liquid (52 mg), bp 85°/18 mmHg; $[\alpha]_D$ +27.8° (c 5.0, MeOH); IR (neat): 2990, 2960, 1732, 1116, 1045 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, d, J=7.2 Hz, 2-CH_3), 1.22 (3H, d, J=6.3, 3-CH_3), 2.47 (1H, dq, J=7.2, 7.2 Hz, 2-H), 3.81 (3H, s, $COOCH_3$), 3.88 (1H, m, 3-H).

The aqueous phase was extracted with n-BuOH (100 ml) and the organic phase was evaporated in vacuo to yield a mixture of glycosidic acid, solid (12.2 g). It (4.2 g) was chromatographed on Si-

gel ($CHCl_3$-MeOH-H_2O, 7:3:0.5) to give **5**, white powder (3.0 g), which was identical with **5** by ^{1}H-NMR and IR, and a white powder (360 mg), mp 122-125° (dec); ^{13}C-NMR (pyridine-d_5) δ: 102.6 (d, fuc-C_1), 77.8 (d, fuc-C_2), 76.0 (d, fuc-C_3), 72.9 (d, fuc-C_4), 70.8 (d, fuc-C_5), 17.1 (q, fuc-C_6), 102.0 (d, glc-C_1), 79.0 (d, glc-C_2), 78.9 (d, glc-C_3), 72.6 (d, glc-C_4), 76.9 (d, glc-C_5), 63.1 (t, glc-C_6), 101.7 (d, rha-C_1), 71.9 (d, rha-C_2), 72.3 (d, rha-C_3), 85.1 (d, rha-C_4), 67.8 (d, rha-C_5), 18.7 (q, rha-C_6), 106.0 (d, qui-C_1), 76.4 (d, qui-C_2), 78.1 (d, qui-C_3), 76.6 (d, qui-C_4), 73.0 (d, qui-C_5), 18.5 (q, qui-C_6).

Acidic Hydrolysis of Glycosidic Acid Fraction

A solution of the glycosidic acid fraction (11.2 g) in 1,4-dioxane-H_2O (1:3) (50 ml) was heated with 10 % H_2SO_4 (50 ml) under reflux for 2h. The mixture was shaken with ether, and the ether layer was concentrated. The residue was column chromatographed over Si-gel (n-hexane-AcOEt, 3:1) followed by crystallization from MeOH to provide **8**, colorless needles (2.1 g), mp 63°; EI-MS m/z: 272 (M^+); Rf 0.60 ($CHCl_3$-MeOH, 8:2). Compound **8** was shown to be identical with jalapinolic acid[12b] (mixed mp, TLC).

Compound **8** (2 g) was methylated with diazomethane followed by crystallization from petroleum ether to afford **9**, yellowish plates (2.1 g), mp 42.5-43.3°; $[\alpha]_D$ +1.0° (c 10.0, $CHCl_3$); EI-MS m/z: 286 $(M)^+$, 255 $(M-OCH_3)^+$, 215 $(CH(OH)(CH_2)_9COOCH_3)^+$, 101 $(CH_3(CH_2)_4CH(OH))^+$; IR (KBr): 3360 (OH), 1745 (C=O), 1170 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t, J=7.0 Hz, 16-H_3), 2.30 (2H, d, J=7.0 Hz, 2-H), 3.66 (3H, s, J=7.0 Hz, $COOCH_3$); Rf 0.26 (solv. 3); (Found: C, 71.0; H, 12.0. Calc. for $C_{17}H_{34}O_3$: C, 71.3; H, 12.0 %). Compound **9** was identified with authentic methyl jalapinolate[12b] (^{1}H-NMR, IR).

The aqueous layer was neutralized with $BaCO_3$ and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure to afford a syrup (7.5 g), which was separated by Avicel SF column chromatography (H_2O sat. n-BuOH) to give four products (all as resinous powder), D-fucose (200 mg), $[\alpha]_D$ +74.3° (c 2.0, H_2O); Rf 0.47(silica gel HPTLC (solv. 4), D-glucose (200 mg), $[\alpha]_D$ +53.0° (c 2.0, H_2O); Rf 0.22, L-rhamnose (32 mg), $[\alpha]_D$ +8.5° (c 2.0, H_2O); Rf 0.49 and D-quinovose (40 mg), $[\alpha]_D$ +28.6° (c 2.0, H_2O), Rf 0.48.

Determination of the Absolute Configuration of Methyl Jalapinolate (9) (Horeau's method)[13]

A solution of (±)-2-phenylbutyric anhydride (0.8 g), methyl jalapinolate (**9**) (0.25 g) and pyridine (2 ml) in benzene (15 ml) was kept at 5° for 13 h and then water (15 ml) was added. After stirring for 30 min at room temperature the mixture was acidified by addition of 4 N HCl (15 ml) and extracted with ether. The ether layer was washed with water and shaken with 5 % $NaHCO_3$ (40 ml). The aqueous layer was acidified with 5 N HCl, extracted with ether and the ether layer was dried ($MgSO_4$) and evaporated in vacuo to give 2-phenylbutyric acid (0.30 g) which showed $[\alpha]_D$ + 0.46° (c 8.5, benzene). The IR spectrum was identical to that of authentic 2-phenylbutyric acid.

Permethylation and Methanolysis of 5

Compound **5** was methylated according to Hakomori's method,[16] and the permethylate was methanolyzed by usual way. The product was analyzed by GC. Condition 1 (3 % OV-17 on chromosorb W. (60-80 mesh), 4 mm i.d. x 2 m glass column; column temperature 115°; N_2 2 kg/cm^2) t_R (min): 2.5, 3.5 (methyl 2,3,4-tri-O-methyl-quinovopyranoside), 5.2 (methyl 2,3-di-O-methyl-rhamnopyranoside), 6.5, 8.4 (methyl 3,4-di-O-methyl-fucopyranoside), 14.6 (methyl 3,4,6-tri-O-methyl-glucopyranoside); condition 2 (1 % OV-1 on chromosorb W. (80-100 mesh), 4 mm i.d. x 2 m glass column; column temperature 210°; N_2 1.68 kg/cm^2) t_R (min): 8.1 (methyl jalapinolate).

Partial Acidic Hydrolysis of 5

To a solution of **5** (220 mg) in H_2O-1,4-dioxane (3:1) (15 ml) was added 1 % H_2SO_4 (15 ml) and the mixture was heated on a boiling water bath for 45 min. The solution was neutralized with 2 N KOH, extracted with n-BuOH and the solvent was evaporated in vacuo. Chromatographic separation of the residue (38 mg) over Si-gel ($CHCl_3$-MeOH-H_2O, 9:1:0.1) afforded **10**, white powder (18 mg), mp 89-

93° (dec); $[\alpha]_D$ -4.9° (c 0.4, MeOH); negative ion FAB-MS m/z: 579 (4.5) $(M-H)^-$, 417 (1.0), 271 (0.7); Rf 0.21 (solv. 1); (Found: C, 54.2; H, 9.3 Calc. for $C_{28}H_{52}O_{12}\cdot 2H_2O$: C, 54.5; H, 9.2 %); ^{1}H-NMR: see Table I.

Acidic Hydrolysis of **10**

A solution of **10** (5 mg) in 1,4-dioxane-H_2O (1:1) (5 ml) and 10 % H_2SO_4 (10 ml) was heated under reflux for 2h, then neutralized with $BaCO_3$. After removal of precipitates the filtrate was extracted with ether and the organic layer was evaporated to give jalapinolic acid (1 mg). The aqueous layer was concentrated in vacuo to afford a syrup which was examined by Si-gel HPTLC (solv. 5), Rf 0.10 (D-glucose), 0.29 (D-fucose).

Partial Deacylation of **1**

Compound **1** (500 mg) in triethylamine-MeOH (1:6) (10 ml) was left standing for 30 min at room temperature. The mixture was made acidic (pH 4.0), diluted with water and then extracted with ether. The organic layer was reduced and subjected to preparative HPLC ($CHCl_3$-MeOH-H_2O, 9:2:0.1) to give **11** (12 mg, 2.4 %), **12** (11 mg, 2.2 %) and **13** (41 mg, 9.0 %) along with **1** (410 mg). **11**: colorless needles, mp 122-131° (dec); $[\alpha]_D$ -7.7° (c 0.2, MeOH); (Found: C, 56.9; H, 8.3. Calc. for $C_{55}H_{92}O_{23}\cdot 2H_2O$: C, 57.0; H, 8.3 %); negative ion FAB-MS m/z: 1119 (0.2) $(M-H)^-$, 1019 (0.4), 789 (0.1), 679 (0.2), 661 (0.2), 617 (0.6), 561 (1.2), 417 (1.8), 271 (1.1); Rf 0.63 (solv. 1). **12**: white powder, mp 131-136° (dec), $[\alpha]_D$ -7.0° (c 0.4, MeOH); (Found: C, 57.0; H, 8.2. Calc. for $C_{55}H_{92}O_{23}\cdot 2H_2O$: C, 57.0; H, 8.3 %); negative ion FAB-MS m/z: 1119 (0.3) $(M-H)^-$, 1019 (1.1), 789 (0.1), 679 (0.6), 661 (0.7), 617 (1.2), 561 (0.7), 417 (1.6), 271 (1.1); Rf 0.62 (solv. 1). **13**: colorless needles, mp 121-136° (dec); (Found: C, 56.0; H, 8.2. Calc. for $C_{50}H_{84}O_{22}\cdot 2H_2O$: C, 56.0; H, 8.3 %); negative ion FAB-MS m/z: 1035 (0.4) $(M-H)^-$, 935 (1.1), 789 (0.3), 679 (0.5), 661 (0.5), 617 (1.6), 561 (0.9), 417 (2.6), 271 (1.7). Rf 0.28 (solv. 1). ^{1}H-NMR data of **11**, **12** and **13** are shown in Table I.

Mild Deacylation of **13**

Compound **13** (90 mg) in 7 % NH_3 (MeOH-H_2O, 1:1) (20 ml) was left standing for 1.5 h at room temperature. The mixture was neutralized with 1 N HCl and diluted with water and extracted with ether. The solvent was distilled off in vacuo and the residue was subjected to preparative HPLC ($CHCl_3$-MeOH, 10:3.5) to yield **14** (6 mg, 6.7 %), **15** (5 mg, 6.1 %) and **16** (1 mg, 1.2 %) together with **13** (71 mg). **14**: white powder, mp 90-96° (dec); $[\alpha]_D$ -22.2° (c 0.3, MeOH); negative ion FAB-MS m/z: 1067 (0.2) $(M-H)^-$, 967 (0.1), 617 (0.3), 417 (0.6); Rf 0.24 (solv. 1); (Found: C, 56.0; H, 8.2. Calc. for $C_{51}H_{88}O_{23}\cdot H_2O$: C, 56.3; H, 8.3 %). **15**: white powder, mp 151-156° (dec); $[\alpha]_D$ -6.6° (c 0.6, MeOH); negative ion FAB-MS m/z: 935 (1.1) $(M-H)^-$, 835 (1.1), 689 (0.4), 579 (2.2), 561 (4.5), 417 (3.5), 271 (2.0); Rf 0.20 (solv. 1); (Found: C, 56.5; H, 8.2. Calc. for $C_{45}H_{76}O_{20}\cdot H_2O$: C, 56.6; H, 8.2 %). **16**: white powder, mp 93-94° (dec); negative ion FAB-MS m/z: 967 (0.5) $(M-H)^-$, 885 (0.8), 739 (0.1), 593 (0.5), 579 (0.2), 417 (0.3), 271 (0.2); Rf 0.14 (solv. 1). ^{1}H-NMR data of **14**, **15** and **16** were shown in Table I.

Partial Deacylation of **2**

Compound **2** (510 mg) was deacylated and worked up in the same way as for **1** to give **4** (13 mg, 2.5 %), **17** (10 mg, 2.0 %) and **13** (30 mg, 6.5 %) together with **2** (440 mg). **17**: white powder, mp 131-136°(dec); $[\alpha]_D$ -16.4° (c 0.3, MeOH); negative ion FAB-MS m/z: 1105 (0.1), 1005 (0.5), 789 (0.2), 679 (0.2), 617 (1.4), 561 (0.9), 417 (2.8), 271 (2.1); Rf 0.62 (solv. 1); (Found: C, 56.6; H, 8.2. Calc. for $C_{54}H_{90}O_{23}\cdot 2H_2O$: C, 56.7; H, 8.3 %); ^{1}H-NMR: see Table I.

Partial Deacylation of **3**

Compound **3** (480 mg) was deacylated and worked up in the same way as for **1** to give **18** (10 mg, 2.1 %), **19** (5 mg, 1.0 %) and **13** (21 mg, 4.8 %) together with **3** (400 mg). **18**: colorless needles, mp 112-115° (dec); $[\alpha]_D$ -12.4° (c 0.7, MeOH); negative ion FAB-MS m/z: 1135 (0.1), 1035 (0.2), 789

(0.1), 679 (0.2), 661 (0.8), 617 (1.0), 561 (0.9), 417 (2.5), 271 (1.8); Rf 0.56 (solv. 1); (Found: C, 55.7; H, 8.3. Calc. for $C_{55}H_{92}O_{24}\cdot 3H_2O$: C, 55.4; H, 8.3 %); ^{1}H-NMR: see Table I. **19**: white powder, mp 129-134° (dec); $[\alpha]_D$ -14.3° (c 0.4, MeOH); negative ion FAB-MS m/z: 1135 (0.1), 1035 (0.3), 789 (0.3), 679 (0.3), 661 (0.9), 561 (0.9), 417 (2.0), 271 (1.9); Rf 0.55 (solv. 1); (Found: C, 56.1; H, 8.3. Calc. for $C_{55}H_{92}O_{24}\cdot 2H_2O$: C, 56.3; H, 8.2 %); ^{1}H-NMR: see Table I.

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REFERENCES AND NOTES

1) a) J. F. W. Johnston, Phil. Trans., 342 (1840); b) G. A. Kayser, Justus Liebigs Ann. Chem., **51**, 81 (1844); c) W. Mayer, ibid, **95**, 129 (1855).
2) C. Mannich and P. Schumann, Arch. Pharm. Ber. Dtsch. Pharm. Ges., **276**, 221(1938).
3) H. Wagner, "Medicine and Natural Sciences, Chemistry in Botanical Classification", ed. by G. Bendz and J. Santesson, Academic Press Inc., New York and London, 1974, p. 235.
4) F. B. Power and H. Rogerson, J. Chem. Soc. Trans., **101**, 1(1912).
5) E. J. Shellard, Planta Medica, **9**, 141 (1961).
6) N. Kromer, J. Pract. Chem., **57**, 448 (1898).
7) Y. Asahina and T. Akasu, J. Pharm. Soc. Japan, **45**, 779 (1925).
8) L. A. Davies and R. Adams, J. Am. Chem. Soc., **50**, 1749 (1928).
9) a) Y. Asahina and S. Terada, J. Pharm. Soc. Japan, **39**, 821(1919); b) regenerated from Prof. Asahina's copper(II) nilate.
10) M. Hirota, H. Ohigashi, Y. Oki and K. Koshimizu, Agric. Biol. Chem., **44**, 1351 (1980).
11) A. Tai and M. Imaida, Bull. Chem. Soc. Jpn, **51**, 1114 (1978).
12) Authentic methyl jalapinolate was obtained by usual methylation of jalapinolic acid synthesized by Prof. Kawasaki (ref. T. Kawasaki, J. Pharm. Soc. Japan, **70**, 35 (1950)).
13) A. Horeau, Tetrahedron Lett., **1961**, 506; idem, ibid, **1962**, 965; J. P. Guette and A. Horeau, ibid, **1965**, 3040; A. Horeau and H. B. Kagan, Tetrahedron, **20**, 2431 (1964).
14) K. Yoshimoto and Y. Tsuda, Chem. Pharm. Bull., **31**, 4324 (1983); L. Hough and A. C. Richardson, Rodd's "Chemistry of Carbon Compounds", ed. by S. Coffey, Elsevier, Amsterdam, 1969, vol. I_F, p. 382; A. H. Hains, "Adv. Carbohydr. Chem. Biochem.", ed. by W. Pigman and M. L. Wolfrom, Academic Press, Inc., New York and London, 1976, vol. 33, p. 11.
15) The chloroform extractive showed a chromatogram almost identical in regard to resin glycoside, to that of "jalapin" obtained by Mayer's method (extraction by ether). "Jalapin" contains considerable amounts of contaminants such as coloring matters and triglycerides which disturb the subsequent chromatographic separation of the resin glycosides. Therefore, the chloroform extractive was used in place of ether extractive "jalapin".
16) S. Hakomori, J. Biochem (Tokyo), **55**, 255 (1964).