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Studies on a Novel Anthraquinone and Its Glycosides isolated from *Rubia cordifolia* and *R. akane*

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From Radix Rubiae (dried roots of *Rubia cordifolia*), mollugin, 1-hydroxy-2-methyl-9,10-anthraquinone, alizarin, 1,3-dihydroxy-2-ethoxymethyl-9,10-anthraquinone, lucidin primeveroside, ruberythric acid and three new anthraquinones, 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone, 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-(6′-*O*-acetyl)-α-rhamnosyl-(1→2)-β-glucoside and 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-α-rhamnosyl(1→2)-β-glucoside, were isolated. The new anthraquinone glycosides were also obtained from the roots of *R. akane*, as the main chemical constituents of anthraquinone glycosides. The structures were established by various chemical and spectroscopic methods.

Keywords——*Rubia cordifolia*; *Rubia akane*; Rubiaceae; anthraquinone; 2-methyl-1,3-6-trihydroxy-9,10-anthraquinone; anthraquinone glycoside; mollugin; ^{13}C-NMR

In the course of studies on screening tests for antitumor activity of crude drugs and collected plants, we have found that the methanol extract prepared from the dried roots of *Rubia cordifolia* L. (Rubiaceae) showed antineoplastic activity against Sarcoma 180 ascites in mice.[1] Some variation of activity was occasionally observed among commercial sample of the crude drug. Therefore, an investigation of anthraquinones, known to be major constituents of plants of this family,[2] was undertaken to confirm the relationship between anthraquinones, as the main chemical constituents, and antineoplastic activity in the extract of *R. cordifolia*.

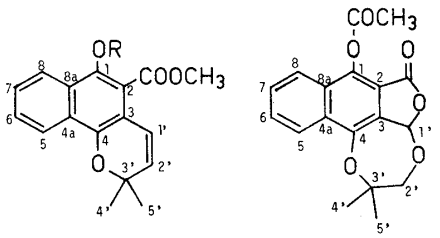
Previous studies on the chemical constituents of *R. cordifolia* collected in India have revealed the presence of anthraquinones,[2,3] prenyl naphthoquinone[4] and triterpenes.[5] However, different types of anthraquinones were isolated from the same plant collected in China. In this paper, we wish to describe the isolation and structure elucidation of these anthraquinones and biosynthetically related compounds.

The methanol extract prepared from the roots of *R. cordifolia* was fractionated by partitioning between benzene and water, and then between ethyl acetate and water. The benzene extract, on column chromatography over silica gel and Sephadex LH-20, yielded compounds I—V. The ethyl acetate extract, on chromatography over Amberlite XAD-2 and silica gel, furnished compounds VI—VIII. After the previous manner, compound IX was isolated by droplet counter current chromatography (DCCC). Compounds I—IV, VIII and IX were established to be mollugin,[6] 1-hydroxy-2-methyl-9,10-anthraquinone,[7] alizarin,[8] 1,3-dihydroxy-2-ethoxymethyl-9,10-anthraquinone,[9] lucidin primeveroside[10] and ruberythric acid,[11] respectively, by comparison with authentic samples or by comparison of various physical and spectral data with those in the literature. However, since the ^{13}C- nuclear magnetic resonance (^{13}C-NMR) spectrum have been erroneously assigned, we attempted to assign the carbon signals correctly by means of known chemical shift rules,[12] the off-resonance decoupling technique and by comparing the ^{13}C-NMR spectrum of mollugin with those of its derivatives (Ia—c). Compounds Ia and Ib were derived from mollugin in the usual way. Compound Ic was prepared by reducing the ozonide of acetylmollgin (Ia) with $NaBH_4$. The quaternary and carbonyl carbon signals, which were difficult to find in the ^{13}C-NMR

TABLE I. ^{13}C Chemical Shifts of Mollugin and Its Derivatives

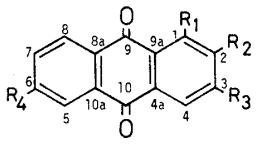
Carbon No.	I	Ia	Ib	Ic
1	156.37 (s)	138.84 (s)	156.07 (s)	140.51 (s)
2	102.11 (s)	119.29 (s)	105.27 (s)	129.21 (s)[b]
3	112.49 (s)	112.66 (s)	111.51 (s)	113.36 (s)
4	141.49 (s)	146.68 (s)	141.43 (s)	144.95 (s)
4a	128.92 (s)	126.56 (s)[a]	128.75 (s)	127.48 (s)[b]
5	123.96 (d)	122.00 (d)	123.67 (d)	123.04 (s)[c]
6	129.27 (d)	127.25 (d)	128.92 (d)	129.09 (d)
7	126.21 (d)	127.25 (d)	125.52 (d)	127.89 (d)
8	121.83 (d)	119.98 (d)	121.48 (d)	122.93 (d)[c]
8a	125.00 (s)	126.85 (s)[a]	125.52 (s)	132.21 (s)
CO	172.39 (s)	169.22 (s)	172.05 (s)	168.47 (s)
OMe	52.24 (q)	52.30 (q)	51.95 (q)	—
Ac-CO	—	165.99 (s)	—	164.84 (s)
Ac-Me	—	20.58 (q)	—	20.53 (q)
1′	122.24 (d)	122.29 (d)	33.21 (t)	100.21 (d)
2′	128.75 (d)	129.90 (d)	23.29 (t)	75.36 (t)
3′	74.61 (s)	76.51 (s)	72.94 (s)	82.51 (s)
4′	26.87 (q)	27.68 (q)	26.47 (q)	25.95 (q)[d]
5′	26.87 (q)	27.68 (q)	26.47 (q)	21.97 (q)[d]

The measurements were made on a JEOL FX-100 spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference and are expressed in terms of ppm.
a—d) The assignments may be reversed.



I: R=H Ia: R=$COCH_3$ Ic
Ib: R=H, $\Delta^{1'}$-hydride

Chart 1



II: R_1=OH, R_2=CH_3, R_3=R_4=H
III: R_1=R_2=OH, R_3=R_4=H
IV: R_1=R_3=OH, R_2=$CH_2OCH_2CH_3$, R_4=H
V: R_1=R_3=R_4=OH, R_2=CH_3
VI: R_1=R_4=OH, R_2=CH_3, R_3=O-(O-6′-Ac)glc$\frac{2\ 1}{}$rham
VII: R_1=R_4=OH, R_2=CH_3, R_3=O-glc$\frac{2\ 1}{}$rham
VIII: R_1=OH, R_2=CH_2OH, R_3=O-glc$\frac{6\ 1}{}$xyl, R_4=H
IX: R_1=OH, R_2=O-glc$\frac{6\ 1}{}$xyl, R_3=R_4=H
X: R_1=R_3=OH, R_2=CH_2OH, R_4=H

Chart 2

spectrum of mollugin, were confirmed by measuring sample solution to which chromium (III) acetylacetonate had been added as a relaxation reagent.[13] These results are shown in Table I.

Compound V, yellowish needles, mp 236—238 °C, mass spectrum (MS) *m/z*: 270 (M^+), exhibited characteristic anthraquinone bands at 1620 and 1590 cm^{-1} in the infrared (IR) spectrum. The ^1H-NMR spectrum showed a singlet signal at δ 2.10 due to an aromatic methyl group, a singlet at δ 7.13 due to an aromatic proton, and a pair of doublets at δ 7.12 and 8.06 (J=8 Hz, in each case) due to two adjacent aromatic protons, one of which (δ 7.12) exhibited long-range coupling with an *m*-positional aromatic proton at δ 7.44 (J=3 Hz). The ^{13}C-NMR spectrum displayed signals assignable to two carbonyl carbons at δ 181.56 and 185.25, one of which was evidently due to a chelated carbonyl carbon, as well as three aromatic hy-

TABLE II. ^{13}C Chemical Shifts of Anthraquinones obtained from *R. cordifolia*

Carbon No.	III	IV	V	VI	VII	VIII	IX	X[a]
1	150.66 (s)	163.63 (s)	162.01 (s)	163.63 (s)	163.57 (s)	161.78 (s)	150.95 (s)	163.4 (s)[i]
2	152.56 (s)	117.05 (s)	117.10 (s)	120.56 (s)	120.56 (s)	123.50 (s)	151.53 (s)	120.1 (s)
3	120.91 (d)[b]	164.03 (s)	162.71 (s)	159.94 (s)	160.17 (s)	161.67 (s)	120.68 (d)[h]	163.0 (s)[i]
4	120.62 (d)[b]	107.65 (d)	106.78 (d)	105.22 (d)	105.05 (d)	106.21 (d)	120.21 (d)[h]	107.7 (d)
4a	123.50 (s)	133.59 (s)	131.46 (s)	135.21 (s)	135.27 (s)	133.54 (s)	125.69 (s)	133.9 (s)[j]
5	126.44 (d)	126.62 (d)[c]	112.20 (d)	112.60 (d)	112.72 (d)	126.73 (d)[f]	126.56 (d)	126.5 (d)[k]
6	134.74 (d)	134.46 (d)[d]	161.67 (s)	161.27 (s)	161.32 (s)	134.51 (d)	134.98 (d)	134.3 (d)[l]
7	133.65 (d)	134.28 (d)[d]	120.85 (d)	121.48 (d)	121.43 (d)	134.51 (d)	134.11 (d)	133.1 (d)[l]
8	126.16 (d)	126.21 (d)[c]	128.98 (d)	129.56 (d)	129.62 (d)	126.33 (d)[f]	126.39 (d)	126.2 (d)[k]
8a	133.31 (s)	132.67 (s)[e]	124.54 (s)	124.31 (s)	124.43 (s)	132.55 (s)[g]	133.13 (s)	131.7 (s)[j]
9	188.43 (s)	185.89 (s)	185.25 (s)	186.18 (s)	186.23 (s)	186.81 (s)	188.20 (s)	185.9 (s)
9a	115.89 (s)	108.86 (s)	108.34 (s)	110.59 (s)	110.58 (s)	111.22 (s)	116.11 (s)	110.6 (s)
10	180.06 (s)	181.44 (s)	181.56 (s)	181.56 (s)	181.56 (s)	181.21 (s)	180.64 (s)	181.5 (s)
10a	132.50 (s)	132.84 (s)[e]	131.46 (s)	131.86 (s)	131.92 (s)	132.67 (s)[g]	132.73 (s)	132.7 (s)[j]
2-Me	—	—	7.90 (q)	8.76 (q)	8.76 (q)	—	—	—
2-CH_2OEt	—	65.21 (t) 59.21 (t) 15.28 (q)	—	—	—	—	—	—
2-CH_2OH	—	—	—	—	—	50.19 (t)	—	51.2 (t)

The measurements were made on a JEOL FX-100 spectrometer in DMSO-d_6 with TMS as an internal reference and are expressed in terms of ppm.

a) The chemical shifts of X are cited from ref. 13.

b—*l*) The assignments may be reversed.

TABLE III. ^{13}C Chemical Shifts of Sugar Moieties in Anthraquinone Glycosides from *R. cordifolia*

	VI		VII		VIII		IX
	Glucose		Glucose		Glucose		Glucose
1′	97.44 (d)	1′	97.50 (d)	1′	100.67 (d)	1′	99.69 (d)
2′	76.45 (d)[a]	2′	76.45 (d)[b]	2′	73.11 (d)	2′	72.88 (d)[e]
3′	77.09 (d)[a]	3′	77.09 (d)[b]	3′	75.65 (d)[d]	3′	75.93 (d)[f]
4′	70.11 (d)	4′	69.59 (d)	4′	69.07 (d)[c]	4′	69.42 (d)
5′	74.09 (d)	5′	77.38 (d)	5′	75.65 (d)[d]	5′	75.93 (d)[f]
6′	63.37 (t)	6′	60.31 (t)	6′	67.86 (t)	6′	68.15 (t)
Ac-Me	20.41 (q)						
Ac-CO	170.26 (s)						
	Rhamnose		Rhamnose		Xylose		Xylose
1″	100.27 (d)	1″	100.21 (d)	1″	103.90 (d)	1″	103.84 (d)
2″	70.46 (d)	2″	70.46 (d)	2″	73.11 (d)	2″	73.28 (d)[e]
3″	70.46 (d)	3″	70.46 (d)	3″	76.22 (d)[d]	3″	76.28 (d)[f]
4″	72.01 (d)	4″	72.01 (d)	4″	69.36 (d)[c]	4″	69.42 (d)
5″	68.56 (d)	5″	68.50 (d)	5″	65.50 (t)	5″	65.44 (t)
6″	18.10 (q)	6″	18.10 (q)				

The measurements were made on a JEOL FX-100 spectrometer in DMSO-d_6 with TMS as an internal reference and are expressed in terms of ppm.

a—*f*) The assignments may be reversed.

droxycarbons at δ 161.67, 162.01 and 162.71, and an aromatic carbon combined with a methyl group at δ 117.10. In addition, signals due to four quaternary aromatic carbons, four tertiary aromatic carbons and one methyl carbon were observed. On the basis of the above results,

compound V was assumed to be 2-methyl-1,3,6(or 7)-trihydroxy-9,10-anthraquinone. However, a detailed comparison of chemical shifts due to the 9- and 10-positional carbonyl carbons in compound V with those in compounds IV and X[14] revealed compound V to be 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone, because the C-10 signals of compounds IV, V and X were at approximately same chemical shift, but the C-9 signal of compound V was shifted 0.6—0.8 ppm upfield with respect to those of compounds IV and X, as a result of transfer of electron density by cross conjugation between C-6 and C-9.[15]

Compound VII, mp 243—245 °C, MS *m/z*: 270 (base peak), was hydrolyzed with 5% HCl to give compound V, glucose and rhamnose. In a ^{13}C-NMR spectral comparison of compounds V and VII, it was apparent that the 3-hydroxyl group in compound VII was substituted with sugar, because the resonance of C-9 in compound VII was shifted 4.7 ppm to lower field in comparison with the C-10 carbonyl signal at δ 181.56, due to strong intramolecular hydrogen bonding between the 1-hydroxyl group and 9-carbonyl oxygen.[15] There was little difference between the chemical shifts of C-6 in compounds V and VII, but the C-3 signal of compound V (at δ 162.71) was shifted upfield to δ 160.17 in compound VII. On the other hand, from a detailed comparison of carbon signals of the sugar moiety in compound VII with those of methyl β-glucose and α-rhamnose,[16] the sugar linkage was concluded to be rhamnosyl (1→2)glucoside, because the resonance of C-2 in the glucose moiety was shifted 2.2—2.8 ppm downfield with respect to the C-2 signal of methyl β-glucose, and the ^{13}C chemical shifts based on the sugar moiety were compatible with those of kaempferol 7-*O*-neohesperidoside (rhamnosyl(1→2)glucoside).[17] On the basis of the above results, compound VII was established as 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-α-rhamnosyl(1→2)-β-glucoside.

Compound VI, yellowish needles, mp 237—238 °C, MS *m/z*: 270 (base peak), was hydrolyzed with 0.2 N NaOH for 5 min to yield compounds V, VII, glucose and rhamnose. The signals at δ 1.95 (3H, s) in the ^{1}H-NMR and at δ 20.41 (q) and 170.26 (s) in the ^{13}C-NMR suggested the presence of an acetyl group. Since the ^{13}C chemical shifts of the aglycone in compound VI were similar to those of compound VII, it was evident that the acetyl group was linked to the sugar moiety. The acetyl group was attached to the 6-positional hydroxyl group of the glucose moiety in the rhamnosyl(1→2)glucoside, because in the ^{13}C-NMR spectral comparison of sugar moieties of compounds VI and VII, the ^{13}C chemical shifts of both rhamnose moieties were approximately the same, but the C-6 signal of the glucose moiety of compound VI was found to appear about 3 ppm downfield from that of compound VII, and the C-5 signal in compound VI appeared at about 3 ppm upfield from that in compound VII. Thus, compound VI was confirmed to be 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-(6′-*O*-acetyl)-α-rhamnosyl(1→2)-β-glucoside.

Compounds VI and VII obtained from *R. cordifolia* were also isolated as main anthraquinone glycosides from the roots of *R. akane*, but they were not detected in the roots of *R. tinctorum*.[18] In our previous studies on the antitumor activity of crude drugs against Sarcoma 180 ascites in mice,[1] the alcohol extracts of *R. cordifolia* and *R. akane* showed antitumor activity, although that of *R. tinctorum* did not. Thus, the relationship between the chemotaxonomy arising from anthraquinones and the antitumor activity in *Rubia* plants seems to be significant.

Experimental

All melting points were recorded on a Yanagimoto micro melting point apparatus and are uncorrected. Spectral data were obtained on the following machines; ultraviolet (UV) on a Shimadzu UV-210, NMR on a JEOL JNM-PS-100 or FX-100, MS on a Hitachi M-80 and IR on a JASCO A-302. An Ohkura GC-103 gas chromatograph equipped with a flame ionization detector was employed for analysis.

Extraction and Isolation——The roots, cut into pieces, of *Rubia cordifolia* used in this experiment were obtained commercially in China. The crude drug (9.5 kg) was extracted with methanol (18 l), three times. The methanol extract (0.8 kg) was partitioned between water (1.5 l) and benzene (1.5 l) with a separatory funnel and then between the water and ethyl acetate (1.5 l). The partitions were each repeated three times, and 350 g of benzene and 250 g of ethyl acetate extracts were obtained. The benzene extract (100 g) was subjected to column chromatography on silica gel. Elution with *n*-hexane–ethyl acetate (95 : 5) gave 1.8 g of mollugin (I). The fraction eluted with *n*-hexane–ethyl acetate (9 : 1) was applied to a Sephadex LH-20 column to yield 40 mg of 1-hydroxy-2-methyl-9,10-anthraquinone (II) by eluting with chloroform–methanol (1 : 1). Elution with *n*-hexane–ethyl acetate (7 : 3) gave 10 mg of alizarin (III) and 110 mg of 1,3-dihydroxy-2-ethoxymethyl-9,10-anthraquinone (IV). Further Sephadex LH-20 column chromatography of the fraction eluted with ethyl acetate–methanol (1 : 1) furnished 50 mg of 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone (V).

The ethyl acetate extract (180 g) was subjected to Amberlite XAD-2 column chromatography and eluted with water, water–methanol and methanol. The fraction eluted with water–methanol (1 : 1) was applied to a silica gel column to yield 0.7 g of 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-(6′-*O*-acetyl)-α-rhamnosyl(1→2)-β-glucoside (VI), 1.4 g of 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-α-rhamnosyl(1→2)-β-glucoside (VII), 120 mg of lucidin primeveroside (VIII) and 90 mg of ruberythric acid (IX) by eluting with a chloroform–methanol solvent system. Further silica gel column chromatography of the fraction eluted with methanol gave 1.9 g of compound VI by eluting with chloroform–methanol (9 : 1).

Physical and spectral data were as follows; I: mp 132—134 °C (from ethanol). *Anal.* Calcd for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.93; H, 5.55. MS *m/z* (%): 284 (M^+, 35), 269 (19), 252 (45), 237 (100), UV λ_{max}^{MeOH} nm (ε): 238 (7100), 247 (7300), 265 (7300), 273 (8300), 282 (8600), 392 (2100). IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.48 (6H, s), 3.97 (3H, s), 5.63 (1H, d, *J*=10 Hz), 7.06 (1H, d, *J*=10 Hz), 7.35—7.65 (2H, m), 8.13 (1H, dd, *J*=8, 2 Hz), 8.31 (1H, dd, *J*=8, 2 Hz), 12.04 (1H, s). II: mp 181—183 °C (from chloroform–methanol). MS *m/z* (%): 238 (M^+, 100), 210 (15), 181 (13). UV λ_{max}^{MeOH} nm (ε): 226 (8100), 254 (11300), 276 (5700), 323 (1350), 400 (1900). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1670 (C=O), 1635 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.30 (3H, s), 7.39 (1H, d, *J*=8 Hz), 7.61 (1H, d, *J*=8 Hz), 7.62—7.78 (2H, m), 8.07—8.27 (2H, m), 12.81 (1H, s). III: mp 198—205 °C (from methanol). MS *m/z* (%): 240 (M^+, 100), 212 (13). UV λ_{max}^{MeOH} nm (ε): 248 (15300), 265 (11900, sh), 275 (9000, sh). IR ν_{max}^{KBr} cm^{-1}: 3250 (OH), 1660 (C=O), 1630 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 7.10 (1H, d, *J*=8 Hz), 7.49 (1H, d, *J*=8 Hz), 7.73—7.87 (2H, m), 7.96—8.11 (2H, m). IV: mp 183—185 °C (from methanol). MS *m/z* (%): 298 (M^+, 9), 269 (7), 252 (100). UV λ_{max}^{MeOH} nm (ε): 240 (1200), 244 (12100), 278 (11000), 406 (2800). ^{1}H-NMR (DMSO-d_6) δ: 1.11 (3H, t, *J*=7 Hz), 3.47 (2H, q, *J*=7 Hz), 4.40 (2H, s), 7.14 (1H, s), 7.77—7.91 (2H, m), 7.95—8.13 (2H, m). V: mp 236—238 °C (from methanol). MS *m/z* (%): 270 (M^+, 100). UV λ_{max}^{MeOH} nm (ε): 284 (42900), 304 (16700, sh), 321 (6600), 424 (6700). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1660 (C=O), 1620 (C=O), 1590 (aromatic C=C). ^{1}H-NMR (CD_3OD) δ: 2.10 (3H, s), 7.12 (1H, dd, *J*=8, 3 Hz), 7.13 (1H, s), 7.44 (1H, d, *J*=3 Hz), 8.06 (1H, d, *J*=8 Hz). VI: mp 237—238 °C (from methanol). MS *m/z*: 270 (base peak). UV λ_{max}^{MeOH} nm (ε): 273 (18000), 305 (5100), 400 (2600). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1720 (C=O), 1660 (C=O), 1630 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, d, *J*=6 Hz), 1.95 (3H, s), 2.16 (3H, s), 5.28 (1H, d, *J*=2 Hz), 5.42 (1H, d, *J*=7 Hz), 7.18 (1H, dd, *J*=8, 3 Hz), 7.37 (1H, s), 7.44 (1H, d, *J*=3 Hz), 8.04 (1H, d, *J*=8 Hz). VII: mp 243—245 °C (from methanol). MS *m/z*: 270 (base peak). UV λ_{max}^{MeOH} nm (ε): 272 (19200), 304 (5900), 408 (6000). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1670 (C=O), 1630 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, d, *J*=6 Hz), 2.19 (3H, s), 5.32 (1H, d, *J*=2 Hz), 5.48 (1H, d, *J*=7 Hz), 7.27 (1H, dd, *J*=8, 3 Hz), 7.44 (1H, s), 7.52 (1H, d, *J*=3 Hz), 8.13 (1H, d, *J*=8 Hz). VIII: mp 210—212 °C (from methanol). MS *m/z*: 270 (base peak). UV λ_{max}^{MeOH} nm (ε): 242 (17400), 260 (18200), 264 (18200), 400 (3900). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1670 (C=O), 1630 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 4.67 (2H, s), 4.24 (1H, d, *J*=6 Hz), 5.13 (1H, d, *J*=7 Hz), 7.51 (1H, s), 7.92—8.05 (2H, m), 8.17—8.31 (2H, m). IX: mp 266—269 °C (from methanol). MS *m/z*: 240 (base peak). UV λ_{max}^{MeOH} nm (ε): 241, 248, 324, 414. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1665 (C=O), 1635 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 7.68 (1H, d, *J*=8 Hz), 7.80 (1H, d, *J*=8 Hz), 7.93—8.07 (2H, m), 8.18—8.34 (2H, m).

Acetylmollugin (Ia), Dihydromollugin (Ib) and Compound Ic——Acetylmollugin was prepared in the usual way by using anhydrous pyridine and acetic anhydride, dihydromollugin by reducing mollugin with 5% Pd-C/H_2, and compound Ic by reducing the ozonide of acetylmollugin with $NaBH_4$. The physical and spectral data were as follows: Ia: pale yellowish oil, MS *m/z* (%): 326 (M^+, 25), 284 (72), 269 (46), 252 (83), 237 (100), 152 (19). ^{1}H-NMR ($CDCl_3$) δ: 1.52 (6H, s), 2.40 (3H, s), 3.92 (3H, s), 5.66 (1H, d, *J*=10 Hz), 6.58 (1H, d, *J*=10 Hz), 7.42—7.55 (2H, m), 7.61—7.74 (1H, m), 8.09—8.23 (1H, m). Ib: mp 101—102 °C. MS *m/z* (%): 286 (M^+, 32), 254 (100), 198 (61), 170 (35). ^{1}H-NMR ($CDCl_3$) δ: 1.38 (6H, s), 1.80 (2H, t, *J*=7 Hz), 3.02 (2H, t, *J*=7 Hz), 3.94 (3H, s), 7.34—7.64 (2H, m), 8.14 (1H, dd, *J*=8, 2 Hz), 8.32 (1H, dd, *J*=8, 2 Hz), 12.14 (1H, s). Ic: mp 206—208 °C, *Anal.* Calcd for $C_{18}H_{16}O_6$: C, 65.88; H, 4.91. Found: C, 65.57; H, 4.89. MS *m/z* (%): 328 (M^+, 2), 286 (32), 215 (100), 214 (77), 186 (11), 158 (23), 43 (47). ^{1}H-NMR ($CDCl_3$) δ: 1.17 (3H, s), 1.59 (3H, s), 2.54 (3H, s), 4.03 (2H, s), 6.30 (1H, s), 7.51—7.75 (2H, m), 7.98—8.31 (2H, m).

Hydrolysis of Anthraquinone Glycosides——Compound VI (2 mg) was hydrolyzed with 0.2 N NaOH (2.0 ml) in water–methanol (1 : 1), for 5 min at room temperature, and compounds V and VII were detected on thin layer chromatography (TLC) (*Rf* value of V, 0.42; VII, 0.08; 0.25 mm Silica gel plate $60F_{254}$ (Merck); lower layer of

$CHCl_3$–MeOH–H_2O (35:65:40)). Compound VI (5 mg) was also hydrolyzed with 5% H_2SO_4 (5.0 ml) for 5 h at 70 °C, then cooled. The reactant was extracted with ethyl acetate (10 ml × 3). The ethyl acetate layer was dried and concentrated to give compound V (1.5 mg), whose identity was confirmed by IR, MS and TLC. After neutralization of the mother liquor with 1 N NaOH, the aqueous solution was applied to Amberlite IR 120B and IR 4B columns and the eluate was evaporated to dryness. The residue was trimethylsilylated and applied to gas liquid chromatography (GLC), which showed the presence of glucose and rhamnose (GLC conditions: 1.5% silicone SE-30, 3 mm × 2 m, column temp. 150 °C, N_2 1.3 kg/cm^2; t_R values, rhamnose 2.0 and 2.7 min, glucose 4.3 and 7.1 min).

The roots (500 g) of *R. akane* collected at Tsukui in Kanagawa Prefecture furnished 680 mg of 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-(6′-*O*-acetyl)-α-rhamnosyl(1→2)-β-glucoside (VI) and 190 mg of 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*-α-rhamnosyl(1→2)-β-glucoside (VII) when subjected to a similar isolation procedure.

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