[Chem. Pharm. Bull. 17(11)2223—2229(1969)] UDC 581.19 : 547.597.02 : 582.734.4

Studies on Constituents of Medicinal Plants. IX.[1] A Constituent of the Roots of *Rosa multiflora* THUNB.

KŌTARO TAKAHASHI, MASARU OGURA
and YOSHIHISA TANABE

Faculty of Pharmaceutical Sciences, Kanazawa University[2]

(Received December 6, 1968)

A triterpenoid $C_{30}H_{48}O_5$, mp 266—267°, was isolated from the roots of *Rosa multiflora* THUNB., and was proved to be identical with tormentic acid (2α, 18α-dihydroxyursolic acid). Plausible mass spectral fragmentations of the diacetate (II) were discussed.

There have been published some papers regarding the structural elucidation of constituents of *Rosa multiflora* THUNB. Kondo[3] and Aritomi[4] isolated multiflorin and astragalin from the fruits and the flower petals respectively, and assumed their structures.

For some time, we have been interested in constituents of the roots of the plant and have isolated a colorless crystalline compound of mp 266—267°. (I). Spectral and chemical evidences of the compound have been accumulated to elucidate the structure. Microanalyses suggested the molecular formula of the compound to be $C_{30}H_{48}O_5$. Infrared (IR), ultraviolet (UV) and nuclear magnetic resonance (NMR) spectral analyses (see the experimental section) suggested the presence two secondary OH groups, a tertiary OH group, a COOH group and a trisubstituted double bond in the compound. We have eventually assumed in the preliminary report published in early 1967 the structure to be a dihydroxyursolic acid. It was some time around the publication of the preliminary report[5] that we recognized that a French group led by Dr. P. Potier had isolated a triterpenoid named as tormentic acid[6] from the roots of *Potentilla tormentilla*, and that physical properties of tormentic acid seemed to be almost identical with those of the compound (I) we have isolated. Further chemical and spectral data of diacetyl derivative (II) of the compound (I) convinced us that these two compounds would be identical.

TABLE I

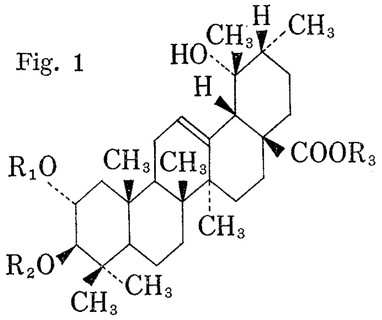
	The triterpenoid	Tormentic acid
Acid	mp 266—267°	mp 273°
Di-acetate	mp 194°	mp 193°
	$[\alpha]_D^{20}=+14°$ (c=1.0 EtOH)	$[\alpha]_D=+12$ (c=1)
Diacetylmethyl ester	mp 163°	mp 162°
Methyl ester	mp 157°	mp 156°

Recently, Dr. Potier was so kind as to send us a specimen of diacetyltormentic acid, which was finally proved to be identical with the diacetyl compound (II) on mixed mp determination.

---

[1] Part VIII: K. Takahashi and T. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 641 (1966).
[2] Location: *Takaramachi, Kanazawa.*
[3] H. Kondo, K. Iwamoto and Y. Kuchiha, *Yakugaku Zasshi*, **49**, 232 (1929).
[4] M. Arotomi, *Yakugaku Zasshi*, **82**, 771 (1962).
[5] K. Takahashi, *Rep. Res. Minist. Educ. Med.*, (1), 252 (Feb. 1967).
[6] P. Potier, B.C. Das, Anne-Marie Bui and M.M. Jonot, *Bull. Soc. Chim. Fr.*, **11**, 3458 (1966).

Fig. 1



I: $R_1=R_2=R_3=H$
II: $R_1=R_2=Ac$, $R_3=H$
III: $R_1=R_2=Ac$ $R_3=CH_3$
IV: $R_1=R_2=H$, $R_3=CH_3$

The present paper deals with our experimental data of isolation of the constituent (I) from the roots of *Rosa multiflora* and of characterisation of the compound (I) and its corresponding acetate (II), acetate methyl ester (III) and methyl ester (IV).

The paper also deals with the mass spectral analysis of the compound (II) in detail. The mass spectral data (Fig. 2 and Table II) appeared to be of particular value to elucidate the structure, and led us to postulate tentatively fragmentation patterns of the compound (II) with a triterpenoid skeleton with dihydroxy functions at C_2 and C_3 positions in ring A as follows.

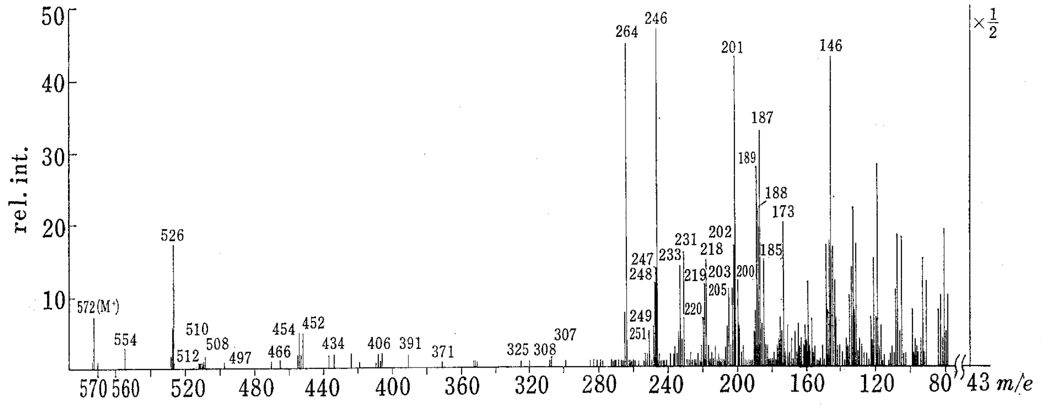


Fig. 2. The Mass Spectrum of Diacetate (II)

Table II

Species	m/e	Relative intensities	Formula	Found	Calcd.	Error (milli-mass units)	Species	m/e	Relative intensities	Formula	Found	Calcd.	Frror (milli-mass units)
M⁺	572	7	$C_{34}H_{52}O_7$	0.371	0.371	0	f	248	12	$C_{16}H_{24}O_2$	0.172	0.178	−6
T	554	3	$C_{34}H_{50}O_6$	0.361	0.361	0	g	247	14	$C_{16}H_{23}O_2$	0.166	0.170	−4
U	526	17	$C_{33}H_{50}O_5$	0.365	0.366	−1	G	246	47	$C_{16}H_{22}O_2$	0.162	0.162	0
S	512	1	$C_{32}H_{48}O_5$	0.346	0.350	−4	j	233	14	$C_{15}H_{21}O_2$	0.153	0.154	−1
b	510	1	$C_{33}H_{50}O_4$	0.369	0.371	−2	K	231	16	$C_{15}H_{19}O_2$	0.136	0.139	−3
c	508	2	$C_{33}H_{48}O_4$	0.354	0.355	−1	F	220	7	$C_{15}H_{24}O$	0.186	0.183	+3
V	497	1	$C_{31}H_{45}O_5$	0.325	0.327	−2	D	219	12	$C_{15}H_{23}O$	0.176	0.175	+1
e	466	1	$C_{31}H_{46}O_3$	0.349	0.345	+4	H	218	10	$C_{15}H_{22}O$	0.169	0.176	+2
d	454	5	$C_{29}H_{42}O_4$	0.310	0.308	+2	M	218	5	$C_{14}H_{18}O_2$	0.132	0.131	+1
X	452	5	$C_{30}H_{44}O_3$	0.331	0.329	+2	I	205	11	$C_{14}H_{21}O$	0.166	0.159	+7
Z	434	2	$C_{30}H_{42}O_2$	0.316	0.318	−2	N	203	11	$C_{14}H_{19}O$	0.140	0.144	−4
Y	406	6	$C_{29}H_{42}O$	0.325	0.324	+1	J	202	17	$C_{15}H_{22}$	0.169	0.172	−3
a	391	2	$C_{28}H_{39}O$	0.302	0.300	+2	L	201	43	$C_{15}H_{21}$	0.161	0.164	−3
m	371	1	$C_{24}H_{35}O_3$	0.255	0.259	−4	O	200	12	$C_{15}H_{20}$	0.159	0.156	+3
n	325	1	$C_{23}H_{33}O$	0.254	0.253	+1	h	189	28	$C_{14}H_{21}$	0.163	0.164	−1
B	308	1	$C_{18}H_{28}O_4$	0.197	0.199	−2	i	188	22	$C_{14}H_{20}$	0.156	0.157	−1
C	307	1	$C_{18}H_{27}O_4$	0.193	0.191	+2	k	187	33	$C_{14}H_{19}$	0.148	0.149	−1
o	307	1	$C_{23}H_{31}$	0.241	0.243	−2	Q	185	15	$C_{14}H_{17}$	0.130	0.133	−3
A	264	45	$C_{16}H_{24}O_3$	0.174	0.173	+1	l	173	20	$C_{13}H_{17}$	0.135	0.133	+2
R	251	5	$C_{15}H_{23}O_3$	0.163	0.165	−2	P	146	43	$C_{11}H_{14}$	0.110	0.110	0
E	249	6	$C_{15}H_{21}O_3$	0.150	0.149	+1		60	17	$C_2H_4O_2$	0.016	0.021	−5
								43	100	C_2H_3O	0.013	0.018	−5

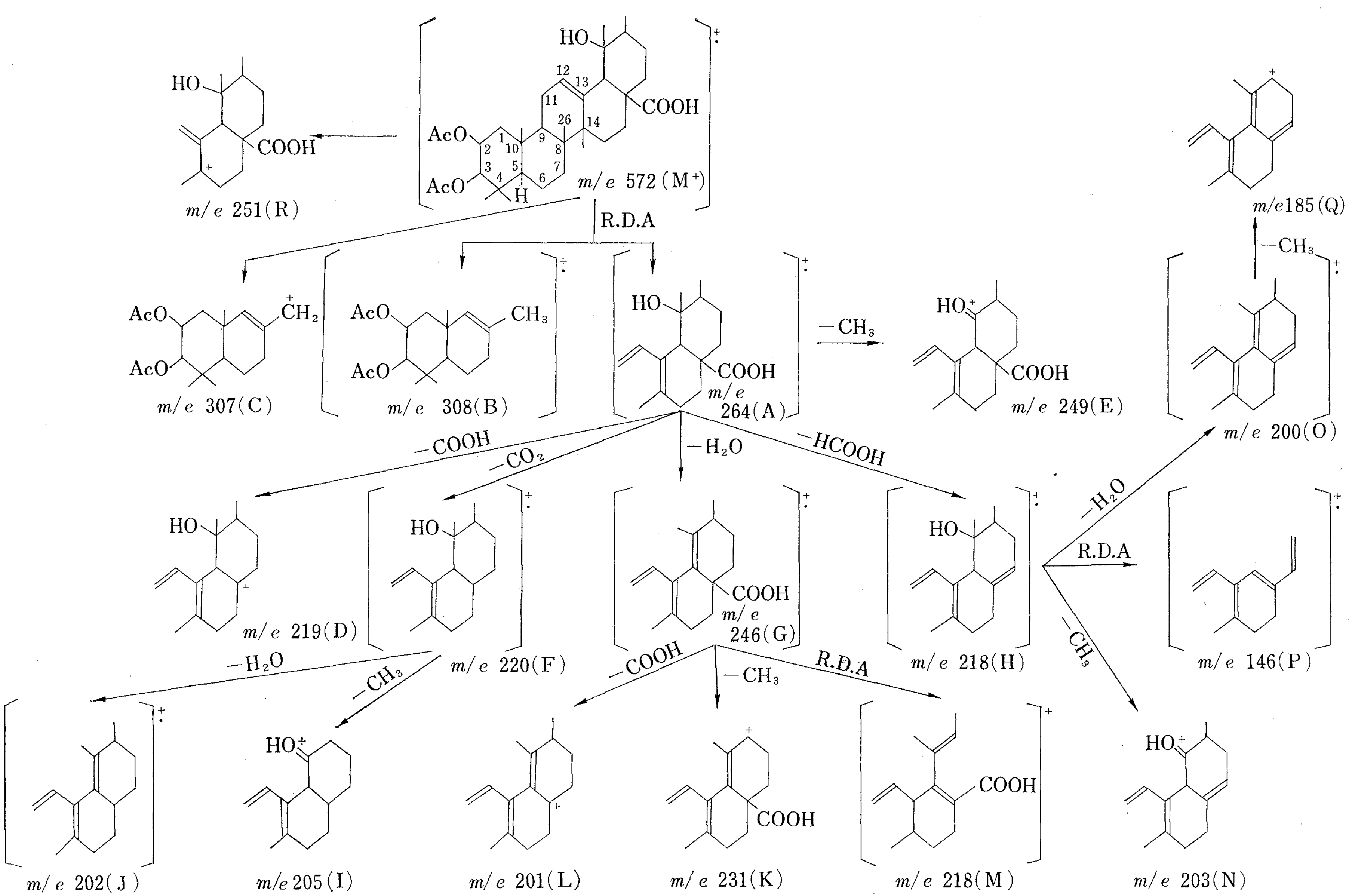

HO
COOH
AcO
AcO
H
1
2
3
4
5
6
7
8
9
10
11
12
13
14
26
m/e 572 (M+)
m/e 251 (R)
R.D.A
m/e 307 (C)
m/e 308 (B)
m/e 264 (A)
−CH3
m/e 249 (E)
m/e 200 (O)
−CH3
m/e185 (Q)
−COOH
−CO2
−H2O
−HCOOH
m/e 219 (D)
m/e 220 (F)
m/e 246 (G)
m/e 218 (H)
−H2O
R.D.A
−CH3
m/e 146 (P)
−H2O
−CH3
−COOH
−CH3
R.D.A
m/e 202 (J)
m/e 205 (I)
m/e 201 (L)
m/e 231 (K)
m/e 218 (M)
m/e 203 (N)

Chart 1

Mass Spectral Analysis

Analysis of peaks in the spectrum of the compound (II) or tormentic acid diacetate was carried out in millimass order. (Fig. 2 and Table II).

The fragmentation by the retro-Diels-Alder (R.D.A.) reaction and the subsequent fragmentations (Chart I and Table II).

The most characteristic fragmentation of II (a Δ^{12}-ursene type triterpenoid) could be described best by the R.D.A. reaction in ring C, the charge remaining with the diene porton *m/e* 264 (A). The charge retention with the other fragment *m/e* 308 (B) could also observed. By

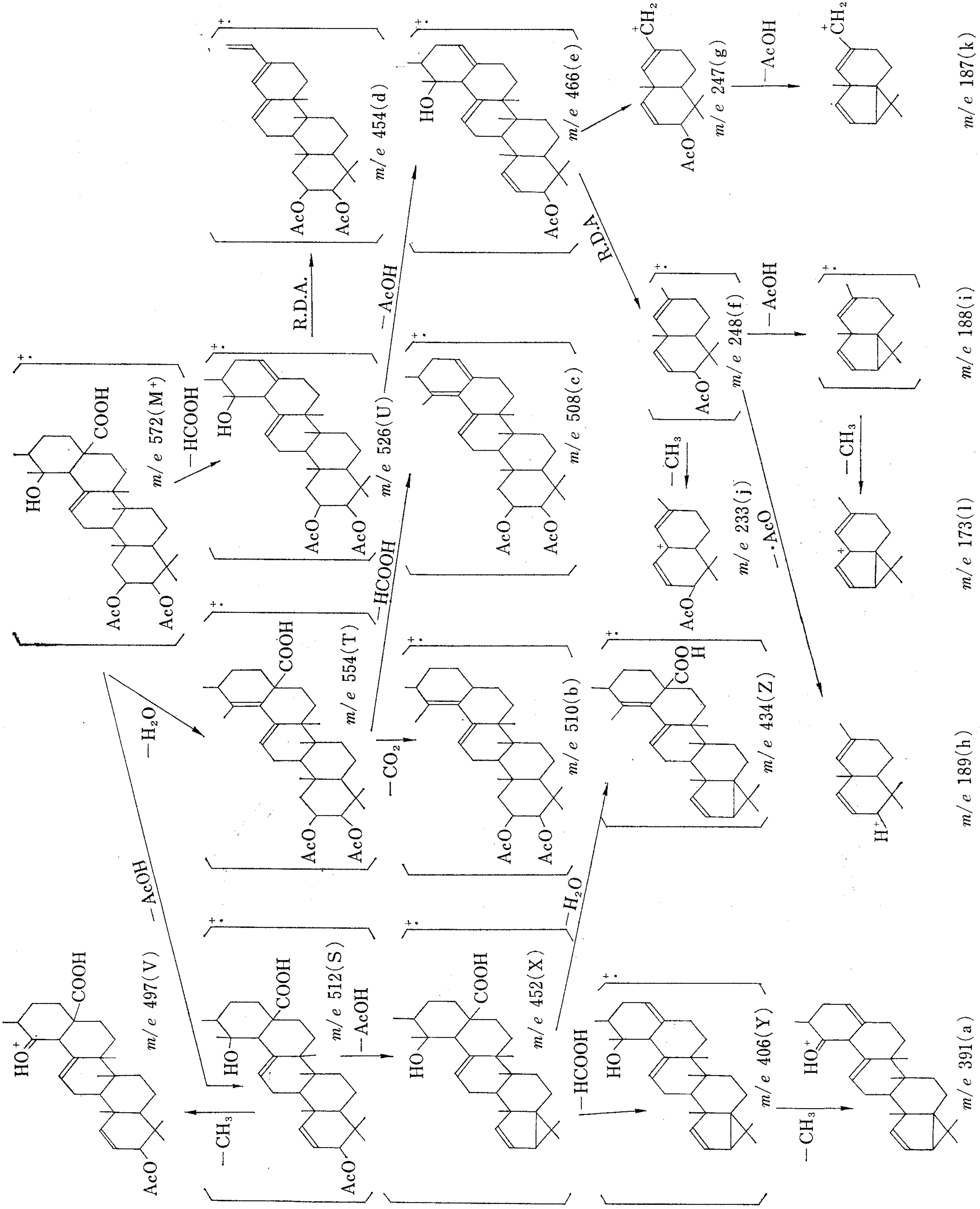


Chart 2

the heterolytic fission of the 8,14-bond activated by an adjascent 12,13-double bond, followed by hydrogen transfer from C_{26} to C_{11} with simultaneous bond breaking at 9—11,[7] II would decompose to yield species *m/e* 307 (C). In this case, the criterion proposed by J. Karliner and C. Djerassi[7] to differentiate Δ^{12}-ursene from Δ^{12}-oleanene by the mass spectrum seems inapplicable (Table II). The species (A) would be subjected to further fragmentations and would decompose by the loss of COOH, CH_3, CO_2, H_2O and HCOOH groups to yield species *m/e* 219 (D), *m/e* 249 (E), *m/e* 220 (F), *m/e* 246 (G) and *m/e* 218 (H), respectively. Species *m/e* 205 (I) and *m/e* 202 (J) would result from species (F) by the loss of CH_3 and H_2O groups, respectively. Species (G) would decompose to yield species *m/e* 231 (K) and *m/e* 201 (L) by the loss of CH_3, and COOH groups, respectively, and also species *m/e* 218 (M) by the R.D.A. reaction in ring E. Species *m/e* 203 (N), *m/e* 200 (O) and *m/e* 146 (P) would result from species (H) by the loss of CH_3, H_2O groups and the R.D.A. reaction in ring E, respectively, and species *m/e* 185 (Q) from species (O) by the loss of CH_3 group. The molecular ion would also decompose to yield species *m/e* 251[8] (R).

The fragmentations of II after the elimination of CH_3COOH, H_2O and HCOOH groups (Chart 2 and Table II). On electron impact, II would decompose, after the loss of CH_3COOH, H_2O and HCOOH groups, to yield species *m/e* 512 (S), *m/e* 554 (T) and *m/e* 526 (U), respectively. Species *m/e* 497 (V) and *m/e* 452 (X) would result from species (S) by the loss of CH_3 and CH_3COOH groups, respectively. Species (X) would decompose to yield species 406 (Y) and *m/e* 434 (Z) by the loss of HCOOH and H_2O groups, respectively, the former fragment of which would give rise to species *m/e* 391 (a) by the loss of CH_3 group. Species (T) would decompose to yield species *m/e* 510 (b) and *m/e* 508 (c) by the loss of CO_2 and HCOOH groups, respectively. Species (U) would decompose to yield species *m/e* 454 (d) and *m/e* 466 (e) by the R.D.A. reaction in the ring E and the loss of CH_3COOH group, respectively, the latter fragment of which would decompose to yield species (H), *m/e* 248 (f) and *m/e* 247 (g) by the mechanism mentioned above. Species (f) would decompose to yield species *m/e* 189 (h), *m/e* 188 (i) and *m/e* 233 (j) by the loss of CH_3COO, CH_3COOH and CH_3 groups, respectively and species (g) would give rise to species *m/e* 187 (k) by the loss of CH_3COOH group and species (i) would give species *m/e* 173 (l) by the loss of CH_3 group.

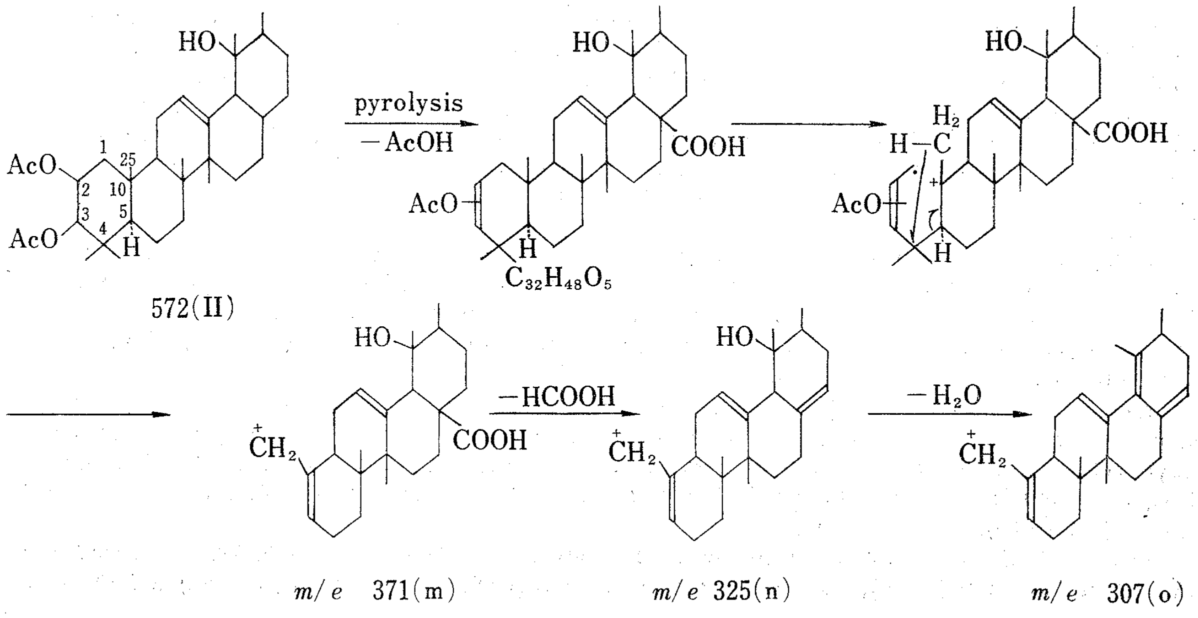


Chart 3

---

[7] J. Karliner and C. Djerassi, *J. Org. Chem.*, **31**, 1945 (1966).
[8] H. Budzikiewicz, J.M. Wilson and C. Djerassi, *J. Am. Chem. Soc.* **85**, 3688 (1963).

The fragmentation involving the fission of ring A. (Chart 3 and Table II).

The diacetate (II) might give $C_{32}H_{48}O_5$ by the loss of CH_3COOH group as the result of pyrolysis, which in turn would decompose to yield species *m/e* 371 (m) on electron impact, by the heterolytic fission of an allylically activated 1—10 bond, followed by a hydrogen transfer from C_{25} to C_4 with simultaneous bond breaking at C_4—C_5. Species (m) would decompose to yield species *m/e* 325 (n) by the loss of HCOOH group and species (n) would give species *m/e* 307 (o) by the loss of H_2O group.

Experimental

Melting points were taken in a Kofler-type hot plate and are uncorrected. The IR spectra were measured in nujol by Nippon Bunko IRS infracode, the UV spectra in EtOH by Hitachi EPS-2U recording spectrophotometer, the NMR spectra in $CDCl_3$ and D_2O by Varian Associates NMR spectrometer at 100 Mc with $(CH_3)_4Si$ as internal reference, and the Mass spectra by JMS-01SG (Mattauch-Herzog type) mass-spectrometer, the ionizing current kept at 200 μA, while the ionizing energy being maintained at 75 eV and the source temperature at 150°.

The Extraction of Constituent from the Roots——Dried powder (1 kg) of the roots of *Rosa multiflora* was suspended in methanol (5 liter) and the suspension was refluxed for 10 hr. The methanolic extract was evaporated *in vacuo* to give a solid precipitate, which was hydrolysed in refluxing 5% ethanolic KOH for 4 hr. The solution was evaporated *in vacuo* to one third volume, poured into ice-water, and acidified with dil. HCl to give a solid. The solid was recrystallized from CH_3OH to give a colorless crystalline powder. The crystalline powder (1 g) was chromatographed over Kieselgel H nach Stahl (100 g) (E. Merck AG) with 15:1 $CHCl_3$-CH_3OH as eluent. The eluate was separated into portions of 200 ml each and the eluate collected from 8th to 12th fractions was evaporated *in vacuo* to give I as a colorless crystalline powder. The powder was recrystallised from CH_3OH to give material of mp 266—267°, wt. 13 mg. *Anal.* Calcd. for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90. Found: C, 73.72; H, 10.03. Liebermann-Burchard test; red to violet; tetranitromethane test; faint yellow. UV: an end absorption at 210 mμ. IR ν_{max} cm^{-1}; 3400 (m, br) (OH), 1695 (s) (COOH), 1640, 825 and 805 (w) (trisubstituted double bond).

Acetylation of I——A solution of I (5 g) in a mixture of pyridine (20 ml) and acetic anhydride (20 ml) was heated on a water-bath for 1 hr, and the solution was poured into ice-water to give a solid precipitate. The precipitate was treated with benzene and the benzene extract was chromatographed over Kieselgel H with 8:2 benzene-ethyl acetate as eluent to give colorless crystals. Recrystallization from benzene gave the diacetate (II) of mp 194° alone, and on admixture: with diacetyltormentic acid.9) *Anal.* Calcd. for $C_{34}H_{52}O_7$: C, 71.29; H, 9.15. Found: C, 71.21; H, 8.87. $[\alpha]_D^{20}$: +14° (c=1.0 EtOH); M^+=572.371 (mass spectrometry); UV: an end absorption at 210 mμ; IR ν_{max} cm^{-1}; 3480 (w) (OH), 1725 (s) (acetate), 1695 (s) (COOH), 1640 (w) (double bond). NMR10) τ:4.73 (one proton, multiplet) (C_{12}-H), 4.83, 4.86, 4.93, 4.96, 5.04 and 5.07 (one proton, sixtet) (C_2-H) (J_{AB}=10 cps, J_{AX}=10 cps, J_{AY}=3 cps), 5.26 and 5.36 (one proton, doublet of AB-type) (C_3-H), 7.49 (one proton, singlet) (C_{18}-H), 7.97 (three protons, singlet) (acetate), 8.04 (three protons, singlet) (acetate), 8.77 and 8.83 (six protons) (C_{29}-CH_3 and C_{30}-CH_3), 8.96 (three protons, singlet) (C_{27}-CH_3), 9.05 (three protons, singlet) (C_{25}-CH_3), 9.11 (six protons, singlet) (C_{24}-CH_3 and C_{23}-CH_3), 9.29 (three protons, singlet) (C_{26}-CH_3).

Methylation of II——The diacetate (II) (400 mg) in ether was methylated with diazomethane as usual and the crude product was chromatographed over Kieselgel H with 16:1 benzene-ethyl acetate as eluent. Recrystallization from petr-ether gave the methylate (III) as colorless crystals of mp 163°. *Anal.* Calcd. for $C_{35}H_{54}O_7$: C, 71.64; H, 9.28. Found: C, 71.89; H, 9.28. IR ν_{max} cm^{-1}: 3480 (w) (OH), 1730 (s) (acetate), 1725 (s) (ester), 1640 (w) (double bond).

Methylation of I——I (1.1 g) in CH_3OH was methylated with diazomethane and the crude methylate was purified by thin-layer chromatography, using a plate (20 × 20 cm) with Kieselgel G (8 g) and 7:3 benzene-ethyl acetate as eluent, giving a colorless crystalline powder (IV) *Rf*=0.18, of mp 157°. *Anal.* Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 74.11; H, 9.85. IR ν_{max} cm^{-1}: 3500—3400 (w, broad) (OH), 1725 (s) (ester), 1640 (w) (double bond).

Characterisation of a 1,2-Glycol Structure in the Methyl Ester (IV)——To a solution of the methyl ester (IV) (37 mg) in EtOH (20 ml), was added 0.02 mole HIO_4 (4 ml) and 10N H_2SO_4 (0.4 ml) and the mixture

---

9) The sample was furnished through the courtesy of Dr. Potier.

10) Tentative assignment of signals of C-methyl groups was based on the data of those methyl groups in acetylursolic acid.11)

11) R. Savoir, R. Ottinger, B. Tursch and G. Chiurdoglu, *Bull. Soc. Chim. Belges.*, **76**, 371 (1967).

kept at room temperature for 10 hr. Consumption of HIO_4 during the period was found to reach 0.87 equivalent.

Acknowledgement The authors are deeply indebted to Dr. P. Potier, Institut de Chemie Substances Naturalles, Centre National de la Recherche Scientique, for the sample of diacetyltormentic acid. The authors wish to thank the Research Laboratory of Takeda Chemical Industries for the measurement of NMR spectra, and to Mr. Y. Ishida and Mr. M. Shindo of Japan Electron Opticus Co., Ltd. fro measuring mass spectra and for valuable discussion on the spectra, and to Miss. S. Kitagawa of the faculty for measuring the rest of mass spectra. Our thanks are due to Mr. M. Ikeda and Mr. M. Kawara of our laboratory for technical assistance and to Mr. Y. Itatani for elemental analyses. A part of the expenses was supported by a Grant-in-Aid of the Ministry of Education, for which the authors wish express their gratitude.