Studies on Iridoid-Related Compounds. V.¹⁾ Antitumor Activity of Iridoid Dervs. Periodate Oxidation Products

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In the course of a modification study of iridoid glucosides to investigate their antitumor and antimicrobial activity, we found that about all metaperiodate oxidation products of iridoid glucosides which had no antitumor activity showed potent activity against the leukemia P388 in mice. They were found to be more active than the corresponding aglycones obtained by enzymic hydrolysis of iridoid glucosides. Among them, periodate oxidation product of sweroside showed the most potent activity, of which the maximum total/control (T/C) value was 198% at 200 mg/kg.

Keywords — iridoid glycoside; iridoid periodate oxidation product; antitumor activity; leukemia P388; iridoid derivative

Introduction

In a previous paper,¹⁾ we reported the antitumor activity of several iridoid glucosides and their aglycones in mice bearing experimental leukemia P388. Most of the aglycones were found to be active. Among them, scandoside methyl ester aglycone showed the most potent activity, and it was further tested against several kinds of experimental tumors. None of the glucosides showed any activity. Our observations suggested that the hemiacetal structure of iridoid aglycones a significant role in the antitumor activity, as in the antimicrobial activity reported previously.²⁾

In a further investigation, we found that periodate oxidation products of iridoid aglycones showed more potent activity than the corresponding aglycones. Here, we wish to report the results of their antitumor activity and elucidation of the structure of the periodate oxidation products of iridoid glucosides.

Materials and Methods

Melting points were measured on a Yanagimoto micromelting point apparatus and their uncorrected values are reported. Infrared (IR) spectra were recorded with a Shimadzu 420 spec-

trometer, ¹H nuclear magnetic resonance (¹H NMR) spectra with a JEOL 200FX spectrometer, ¹³C NMR spectra with a JEOL 100FX (TMS as internal reference) spectrometer, and mass spectra with a JEOL JMS-DX 303 spectrometer.

Iridoid Glucosides (Chart 1) — Aucubin(1), scandoside methyl ester(2), geniposide(3), loganin(4), gardenoside(5), asperuloside(6), catalpol(7), morroniside(8), sweroside(9), 9,10-dihydro sweroside(10) and gentiopicroside(11) were used for oxidation.

1 was isolated from *Aucuba japonica* THUNB., 2 from *Hedyotis corymbosa* LAM., 3 and 5 from *Gardenia jasminoides* ELLIS, 4 from *Cornus officinalis* SIEBOLD *et* ZUCCARINI, 6 from *Daphniphyllum macropodum* MIQ, 8 from *Lonicera japonica* THUNB. and 9 and 11 from *Gentiana scabra* BRUNGE. Each compound was confirmed to be identical with an authentic specimen. 10 was derived from 9 by hydrogenation.

General Procedure for Preparation of Periodate Oxidation Products of Iridoid — Periodate oxidation products were prepared by the oxidation of each iridoid glucosides with a stoichiometric amount of sodium periodate (NaIO₄) at room temperature. The completion of the oxidation was determined by the disappearance of the spot of the starting material on thin layer chromatography (TLC) plates. The

Chart 1

solution was passed through a column of charcoal using H_2O and acetone to remove iodate ion. The iodate ion was checked by a starch-iodide test. The acetone eluate was concentrated and the white powder obtained was characterized by mass spectra, ^{13}C NMR and 1H NMR.

Periodate Oxidation Products (11–22) of Iridoid — **12** from aucubin (**1**); mp 132 °C (dec.), IR ν_{max}^{KBr} cm^{-1} : 3300 (br), 2900, 1640, 1220. EI-MS (20 eV): m/z 314 (M^+),* 166 (aglycone).

13 from scandoside methyl ester (**2**); mp 135–142 °C, IR ν_{max}^{KBr} cm^{-1} : 3350 (br), 2900, 1680, 1630, 1440, 1280. EI-MS (20 eV): m/z 372 (M^+), 354, 225.

14 from geniposide (**3**); mp 114–119 °C, IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3350, 1690, 1652, 1440, 1280. EI-MS (20 eV): m/z 356 (M^+), 372, 208 (aglycone).

15 from loganin (**4**); mp 110–120 °C (dec.), IR ν_{max}^{KBr} cm^{-1} : 3400, 1690, 1630, 1440, 1280. EI-MS (20 eV): m/z 358 (M^+), 211 (aglycone).

16 from gardenoside (**5**); mp 93–97 °C, IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3350, 1705, 1635, 1435. EI-MS (20 eV): m/z 358 ($M^+ + 18$), 340 (M^+).

17 from asperuloside (**6**); mp 103–108 °C, IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3400, 1740, 1660, 1370. EI-MS (20 eV): m/z 383 ($M^+ - 1$), 340, 235 (aglycone), 192 (aglycone- $COCH_3$), 164 (aglycone-

CH_2OAc).

18 from catalpol (**7**); mp 130–133 °C, IR ν_{max}^{KBr} cm^{-1} : 3300, 1650, 1220. EI-MS (20 eV): m/z 330 (M^+).

19 from morroniside-7-OMe (**8**); mp 71–76 °C, IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3400, 1700, 1630, 1435, 1380. EI-MS (20 eV): m/z 388 (M^+), 227.

20 from sweroside (**9**); mp 102–104 °C, IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3500 (br), 1715, 1630. EI-MS (20 eV): m/z 343 ($M^+ + 17$), 327 ($M^+ + 1$), 196 (aglycone), 127.

21 from 9,10-dihydrosveroside (**10**); mp 112–118 °C (dec.), IR ν_{max}^{KBr} cm^{-1} : 3450, 1700, 1680, 1605. EI-MS (20 eV): m/z 329 ($M^+ + 1$).

22 from gentiopicoside (**11**); mp 116–128 °C (dec.), IR ν_{max}^{KBr} cm^{-1} : 3400–3200 (br), 2900, 1700, 1605. EI-MS (18 eV): m/z 324 (M^+).

Animal — Male BDF₁ mice (5 weeks old) were used in the present study. All mice were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animal (Hamamatsu, Shizuoka), and were given pellet diet CE-2 (CLEA Japan, Inc., Tokyo) and water *ad libitum*.

Test Compound — Test compounds were suspended in 0.5% carboxymethylcellulose sodium salt (CMC, Wako Pure Chemical Industries Ltd., Osaka) and administered intraperito-

* M^+ was conveniently adopted for dialdehyde form.

AG = iridoid aglycone

Chart 2

neally in a volume of 0.1 ml/10 g body weight.

Evaluation of Antitumor Activity — Leukemia P388 was inoculated intraperitoneally into BDF₁ mice at 1×10^6 cells/mouse. Each group consisted of 3 mice. The test compounds were injected intraperitoneally on day 1 after tumor inoculation. Antitumor activity was evaluated in terms of the increase in life span over the control. The T/C % value were calculated on the basis of the mean survival time of the treated and control mice.

Test Organisms — Antimicrobial activity was tested against both *Staphylococcus aureus* 209P IFO-3061 and *Escherichia coli* NIHJ.

Antimicrobial Assay — The *in vitro* antimicrobial activity was determined by the agar-well method.^{3a,b)} Plates were prepared to a depth of 4 mm with a Sensitivity test agar(STA: EIKEN) medium which was previously inoculated with 1 percent (v/v) of a test organism grown overnight at 37 °C in Heart infusion broth (HIB: NISSUI). Holes (8 mm diameter) were bored in the medium with a sterile borer, and filled with 40 μ l solution (1000 μ g/ml) of test samples. Plates were sealed and then incubated for 18 h at 37 °C. The activity was expressed by the inhibitory diameter.

Results

Structures of Periodate Oxidation Products of Iridoids

By periodate oxidation, sugar moieties of iridoid glucosides were oxidized. The aglycone moieties remained unreacted except for garadenoside of which the 8,10-glycol moiety was cleaved to 8-ketone. The periodate oxidation of glucoside commonly gives dialdehyde deriva-

tives⁴⁻⁷⁾ and this was the case in iridoid glucoside oxidation by periodate since D/CI MS^{8a,b)} or EI-MS* showed M⁺ for dialdehyde except for **12** (dialdehyde + H₂O in D/CI MS) and **16** (dialdehyde + H₂O in EI-MS). Iridoid periodate oxidation products can exist as various cyclic and acyclic aldehydes (A, C), hemiacetals (B, C, D) and hydrates (D, E) as shown in Chart 2. The aldehyde forms (A or C) can be excluded because there was no evidence for the presence of aldehydic signals in ¹H NMR and ¹³C NMR spectra.

A detailed study of ¹H and ¹³C NMR was made for the periodate oxidation product (**13**) of scandoside methyl ester by means of 360 MHz ¹H NMR using double resonance experiments and a ¹H-¹³C selective decoupling experiment. The ¹H and ¹³C NMR data of **13** are shown in Table I. The two split signals of 1, 3 and 12-H in the aglycone moiety and the presence of the two coupling constants ($J = 2.0$ and 9.5 Hz) of the 4'-H (see Chart 2 for numbering) at 4.44 ppm coupled with 5'-H, J value of the former for an equatorial and the latter an axial proton, suggested that the structure of **13** is an epimeric mixture at C-4' which corresponded to the dihemiacetal B in Chart 2. The configuration at C-2' is not obvious from the coupling constant (0 Hz) between 1'-H and 2'-H but it is note worthy that the dioxane ring of this compound did not have the typical chair conformation.⁹⁾ ¹H NMR spectra of the periodate oxidation products of other iridoid glucosides shown in Chart 1 indicated the same hemiacetal structure as **13**.

Antitumor Activity of Periodate Oxidation Products

The antitumor activity of **12–22** against

* FAB-MS did not show a significant mass fragment.

TABLE I. ^1H and ^{13}C NMR Data for Periodate Oxidation Product of Scandoside Methylester (**13**) in CD_3OD

C/H Number	^1H (ppm)	^{13}C (ppm)
C-1/H-1	5.14 (1/2H, d, $J_{1,9} = 6.5$ Hz) 5.21 (1/2H, d, $J_{1,9} = 6.0$ Hz)	96.77, 96.53 (d)
C-3/H-3	7.53 (1/2H, d, $J_{3,5} = 1.5$ Hz), 7.55 (1/2H, s)	153.82, 153.70 (d)
C-4		111.13 (s)
C-5/H-5	3.05 (1H, m)	46.11, 45.70 (d)
C-6/H-6	4.60 (1H, m, $J_{6,7} = 1.5$, $J_{5,6} = 4.0$ Hz)	82.23, 82.17 (d)
C-7/H-7	5.89 (1H, d, $J_{6,7} = 1.5$, $J_{7,10} = 1.5$ Hz)	130.72 (d)
C-8		147.19 (s)
C-9/H-9	3.05 (1H, m)	47.24 (d)
C-10/H-10a	4.29 (1H, br.d, $J_{7,10} = 1.5$ Hz)	61.17, 61.06 (t)
10b	4.42 (1H, br.d, $J_{10a,b} = 15.0$ Hz)	
C=O		170.11 (s)
OCH_3	3.79 (3/2H, s), 3.80 (3/2H, s ^a)	52.12 (q)
C-1'/H-1'	4.85 (1H, s)	93.90 (d)
C-2'/H-2'	4.73 (1/2H, s), 4.74 (1/2H, s)	90.02 (d)
C-4'/H-4' (eq)	4.44 (1/2H, d, $J_{4',5'} = 2.0$ Hz)	98.16 (d)
(ax)	4.44 (1/2H, d, $J_{4',5'} = 9.5$ Hz)	
C-5'/H-5'	3.80 (1H, m ^a)	70.95, 70.37 (d)
C-6'/H-6'a	3.58 (1H, dd, $J_{6'a,b} = 11.5$ Hz, $J_{6'a,5'} = 10.0$ Hz)	59.19 (t)
H-6'b	4.07 (1H, dd, $J_{6'a,b} = 11.5$ Hz, $J_{6'b,5'} = 2.0$ Hz)	

a) Overlapped.

TABLE II. Antitumor Activity of Iridoid Periodate Oxidation Products on P388

Compounds	T/C %						mg/kg
	10	50	100	200	300	500	
12	103	109	112	134	131		
13	111	129	140	147	158	48	
14	110		130	143			
15			150	150	154		
16	109	63	19				
17	119	117	123	133	30		
18	97	117		126	126		
19	119	117	130	123	58		
20	108	126	140	196	167		
21	112	134	147	132			
22			112		140		

Tumor, P388 (1×10^6 , i.p.). Administration, day 1; i.p.

leukemia P388 is shown in Table II. Almost all these compounds showed significant activities. The maximum T/C values of the periodate oxidation products were: **21**, 147% (at 100 mg/kg); **12**, 134%; **14**, 143%; **17**, 133%; **19**, 123% and **20**

196% (at 200 mg/kg); **13**, 158%; **15**, 154%; **18**, 126%; and **22**, 140% (at 300 mg/kg).

Antimicrobial Activity of Periodate Oxidation Products

None of the **12**–**22** showed any inhibitory ac-

tivity at 1000 $\mu\text{g/ml}$ against Gram positive (*S. aureus*) and Gram negative bacteria (*E. coli*) in contrast to an antimicrobial activity of the corresponding iridoid aglycones.¹⁾

Discussions

The resulting structure of the periodate oxidation of iridoid glucoside (**13**) has an epimeric mixture at C-4' of the dihemiacetal and the configuration at C-2' remains to be assigned with relation to its dioxane ring conformation. The usual dioxane ring exists in the chair conformation⁹⁾ but the periodate oxidation products have a skewed chair conformation because of the absence of coupling between 1'-H and 2'-H. A further conformational analysis of this structure is in progress.

In relation to the structure activity relationship, this dihemiacetal structure must have a significant meaning for the expression of its antitumor activity and not for antimicrobial activity because many iridoid aglycones which have similar hemiacetal structures exhibit antitumor activity as reported previously.¹⁾

Among the compounds reported here, it can be pointed out that, in comparison with the corresponding aglycones for antitumor activities, **12** and **13** showed an increase in therapeutic ratio (optimal dose/the dose showing 30% increase in life span) and **14**, **15**, **19** – **21** and **22** increased the life span as shown in Fig. 1 (a, b and c).

Although the mechanism of action of these compounds remains unknown at present, these activities correlated with the biological activities⁵⁻⁷⁾ of periodate oxidation products of nucleosides and the structural relationship between iridoid aglycones and nucleoside base gives an additional subject for study in this area.

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Fig. 1. Antitumor Activity of Aglycones (EH) and (a) Periodate Oxidation Products (PO: **12**, **13** and **14**) of Iridoid Glucosides (**1**, **2** and **3**), (b) (PO: **15**, **19** and **20**) of Iridoid Glucosides (**4**, **8** and **9**) and (c) (PO: **21** and **22**) of Iridoid Glucosides (**10** and **11**) against Leukemia P388

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