RADIOIMMUNOASSAY OF IRIDOID GLUCOSIDES : PART 1 GENERAL METHODS FOR PREPARATION OF THE HAPTENS AND THE CONJUGATES WITH A PROTEIN OF THIS SERIES OF GLUCOSIDES[1]

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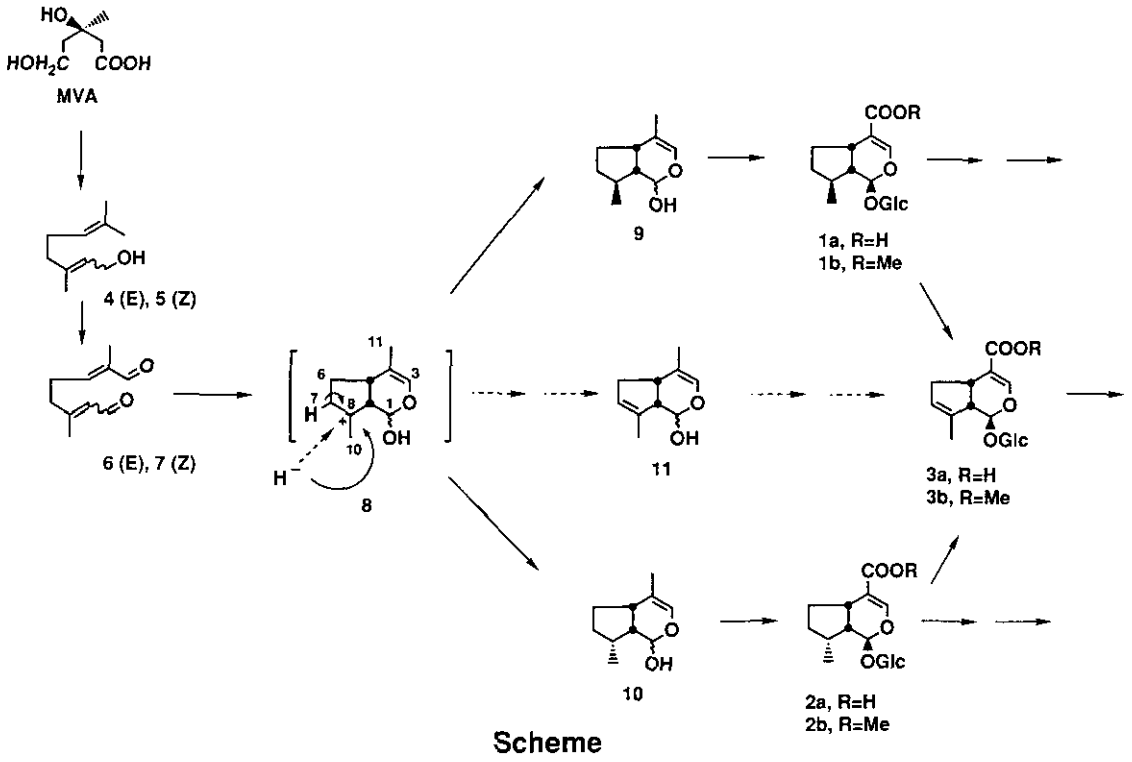
Abstract — In order to obtain antibodies specific to three key intermediates, 7-deoxyloganin (1b), 7-deoxy-8-epi-loganin (2b) and 10-deoxygeniposide (3b), in biosynthesis of iridoid glucosides in behalf of their microdetermination by a radioimmunoassay technique in various plant extracts, we have synthesized, starting with suitable natural iridoid glucosides, three optically pure intermediates from which we in turn prepared their haptens and conjugates to bovine serum albumin.

Though it is known that iridoid glucosides are distributed widely in dicotyledons[2], the iridoid constituents of many plants have not been yet investigated. In fact, during the past decade the number of new glucosides increased rapidly.[3,4,5] The vast majority of these glucosides belong to a group with a carboxy group at C-4 position of iridane as in 7-deoxyloganic acid (1a) or its decarboxylated one which is biosynthetically derived from the former. Their biosynthetic pathway (Scheme) was proposed as follows[6-9]: 1) Three iridodial isomers (9, 10, 11) having the iridane skeleton are formed via cyclization of 10-oxogeranial (6) or its isomer, 10-oxoneral (7), generated through respective oxidation of geraniol (4) or nerol (5) which are derived from mevalonic acid (MVA). 2) These isomers (9, 10, 11) are oxidized followed by glucosidation to be led to three glucosidic intermediates, 7-deoxyloganic acid (1a), 7-deoxy-8-epi-loganic acid (2a) and 10-deoxygeniposidic acid (3a) or their methyl esters (1b, 2b, 3b) (Each free acid and its methyl ester are considered to be biosynthetically equivalent). 3) Each intermediate gives rise to various iridoid glucosides via several oxidation stages.

A cyclization of 6 or 7 provides iridodial cation (8), a supposed intermediate, which in turn forms three biosynthetic intermediates (9, 10, 11) by mode of quenching the cation: the attack

of a hydride to 9 and 10 or the deprotonation to 11. The two intermediates (1b, 2b), of these intermediates, precede 3b in iridoid biosynthesis, because 1b and 2b were incorporated to 3b in plants and their calluses.[8,9] However, the biosynthetic route through compound 11 to 3 is not negligible entirely.

The aim of this study is to investigate through which of these three intermediates (1, 2, 3) iridoid glucosides having a carboxy group at C-4 position present in various plants were biosynthesized and furthermore the distribution of iridoid glucosides in dicotyledons through the detection of these biosynthetic intermediates.



Scheme

As methods for determining a trace amount of a target intermediate in plant extracts, gas chromatographic (GC), GC – mass spectrometric, high performance liquid chromatographic and immunogenic techniques containing radioimmunoassay have been used. In this study, the radioimmunoassay technique was preferred for the determination of iridoid glucosides because of sensitivity and specificity as well as direct applicability to glucosidic extracts. The present paper deals with the preparation of haptens and conjugates with a protein of three optically pure intermediates (1, 2, 3) for establishing their radioimmunoassay system.

Design for the synthesis of immunogenic conjugates

The above-mentioned biosynthetic intermediates (1, 2, 3) of iridoid glucosides could not be immunogenic by themselves because of their low molecular weight. Thus, in order to obtain antibodies against them, it is necessary to conjugate them covalently to an antigenic protein or synthetic polypeptide carrier, before immunization, for which we chose bovine serum albumin (BSA). Moreover, for the preparation of three antibodies specific to three compounds it is better to bind them to BSA at the remote positions from the position to be districtly recognized of their molecule. Thus two types of conjugates with regard to three glucosides (1, 2, 3) were prepared. In the type I (13, 20, 28), glucosides was bound to a BSA through the carboxy group at C-4 position of the aglucone moiety and in the type II (16, 25, 31), through the carboxy group derived from the primary hydroxy group at C-6' position of the sugar moiety. In both cases γ -aminobutyric acid (GABA) was used as a spacer binding two parts.

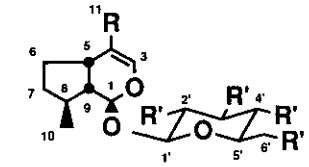
Preparation of six immunogenic conjugates with BSA

In general, a chemically and optically pure substance is necessary for the preparation of the specific antibody against it and for its radioimmunological analysis. Three above-mentioned biosynthetic intermediates (1, 2, 3) of iridoid glucosides are different only in the geometry at C-8 position. So we paid our attention to the preparation of three optically or regiospecifically pure compounds (1, 2, 3).

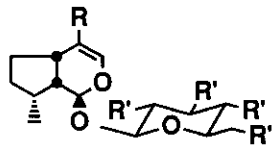
1) 7-Deoxyloganin-BSA conjugates 13 and 16

In the previous studies, 7-deoxyloganin (1b) was prepared through the hydrogenation including hydrogenolysis of geniposide (37)[10] and the acetate (38).[11,12] However, it had been proved from the detailed analysis of the high resolution NMR spectrum that the product contained 5 – 10% of 7-deoxy-8-epi-loganin (2b).

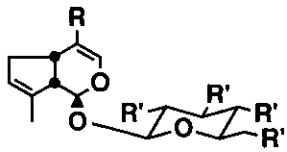
As the starting material for the alternative preparation of 1b, now, verbenalin (39) was chosen because it has 8 β -methyl group which would be unaffected during its conversion to 1b and furthermore is readily obtainable in large quantities from the leaves of Symplocos glauca Koidz. The tetraacetate (40) was treated with ethylenedithiol-BF_3 to give thioketal (41).



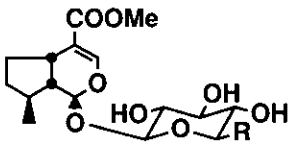
1a. R=COOH, R'=OH
1b. R=COOMe, R'=OH
12. R=COOMe, R'=OAc
13. R=CONH$(CH_2)_3$CO-BSA, R'=OH
14. R=CONH$(CH_2)_3$COOMe, R'=OH
15. R=CONH$(CH_2)_3$COOH, R'=OH



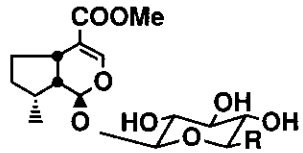
2a. R=COOH, R'=OH
2b. R=COOMe, R'=OH
20. R=CONH$(CH_2)_3$CO-BSA, R'=OH
21. R=CHO, R'=OH
22. R=CHO, R'=OAc
23. R=COOH, R'=OAc
24 R=CONH$(CH_2)_3$COOH, R'=OH



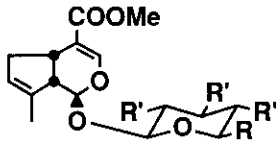
3a. R=COOH, R'=OH
3b. R=COOMe, R'=OH
28. R=CONH$(CH_2)_3$CO-BSA, R'=OH
29. R=COOMe. R'=OAc
30. R=CONH$(CH_2)_3$COOH, R'=OH



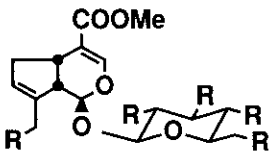
16. R=CONH$(CH_2)_3$CO-BSA
17. R=COOH
18. R=CONH$(CH_2)_3$COOMe
19. R=CONH$(CH_2)_3$COOH



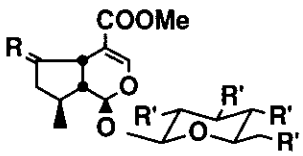
25. R=CONH$(CH_2)_3$CO-BSA
26. R=CONH$(CH_2)_3$COOH
27. R=COOH



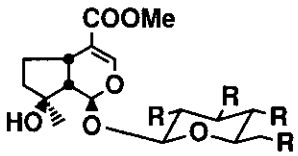
31. R=CONH$(CH_2)_3$CO-BSA, R'=OH
32. R=COOH, R'=OH
33. R=CH_2OTr, R'=OAc
34. R=COOH, R'=OAc
35. R=CONH$(CH_2)_3$COOH, R'=OH
36. R=CONH$(CH_2)_3$COOMe, R'=OAc



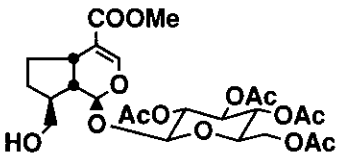
37. R=OH
38. R=OAc



39. R=O, R'=OH
40. R=O, R'=OAc
41. R=-S$(CH_2)_2$S-, R'=OAc



42. R=OH
43. R=OAc



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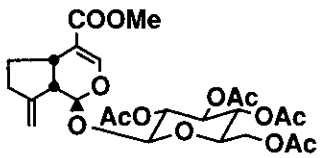

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Figure

Desulfurization of 41 with Raney Ni provided optically pure 7-deoxyloganin tetraacetate (12) which was led to the haptens of types I and II. 7-Deoxyloganic acid (1a) obtained through hydrolysis of 12 was condensed with methyl γ - aminobutyric acid (Me-GABA) using 1-ethyl-3-[3-(dimethyl-amino)propyl]-carbodiimide hydrochloride (EDCI) to afford 11-GABA amide methyl ester (14). Hydrolysis of 14 afforded 11-GABA amide (15), the hapten of type I. On the other hand, 12 was subjected to Zemplen deacetylation[13] to give 7-deoxyloganin (1b). Regioselective oxidation of 1b with platinum oxide in the presence of alkali gave 6'-acid (17) in 75 % yield. In the same way as described for the preparation of 15, 17 was converted, through 6'-GABA amide methyl ester (18), into 6'-GABA amide (19), the hapten of type II. Conjugation of two haptens (15, 19) to BSA with EDCI gave two conjugates, 13 and 16, respectively.

2) 7-Deoxy-8-epi-loganin-BSA conjugates 20 and 25

7-Deoxy-8-epi-loganin (2b) was prepared, as shown below, from boschnaloside (21)[14] in which the methyl group at C-8 position was established to be α -configuration. Oxidation of its acetate (22) with sodium chlorite and sodium dihydrogen phosphate in the presence of 2-methyl-2-butene afforded 7-deoxy-8-epi-loganic acid tetraacetate (23).[15] Methylation of 23 with diazomethane followed by Zemplen deacetylation afforded 7-deoxy-8-epi-loganin (2b).

The two types of 7-deoxy-8-epi-loganin hapten were prepared according to the same procedure as in the above haptens, 15 and 19. 11-GABA amide (24), the hapten of type I, was synthesized via condensation of 23 with Me-GABA followed by hydrolysis. 6'-GABA amide (26), the hapten of type II, was prepared by the regioselective oxidation of 2b with platinum oxide and condensation of the resulting 6'-acid (27) with Me-GABA followed by hydrolysis. Condensation of two haptens (24, 26) with BSA afforded two conjugates 20 and 25, respectively.

3) 10-Deoxygeniposide-BSA conjugates 28 and 31

In the previous works, 10-deoxygeniposide (3b) and its acetate (29) were derived from geniposide (37), mussaenoside (42)[16] and their acetates (38, 43).[17-19] However, hydrogenolysis of geniposide pentaacetate (38) gave a mixture of 29, 12 and 7-deoxy-10-hydroxyloganin tetraacetate (44), besides 38, which were separable in small scale by chromatography on silica gel and subsequently on silica gel impregnated with $AgNO_3$. On the other hand, dehydration of mussaenoside tetraacetate (43) gave 29 containing about 10 % of the 8,10-double bond isomer (45), which were unseparable. However, careful hydrogenolysis of free glucoside 37 in the presence of acid was found to give 3b in high yield which was purified as its acetate (29) by chromatography on silica gel. Glucosides 3a and 3b were obtained through hydrolysis of 29 with 0.4N NaOH and Zemplen deacetylation, respectively. Condensation of 3a with Me-GABA followed by hydrolysis gave 10-deoxygeniposide 11-GABA amide (30), the hapten of type I.

Unfortunately, all attempts to oxidize 3b with platinum oxide to 6'-acid (32) were unsuccessful. Thus we adopted an alternative route leading to 6'-acid (32) or its derivative. 3b was subjected to tritylation with trityl chloride in pyridine but the reaction proceeded very slowly and the yield of the tritylated product also was very low. However, tritylation of 3b with trityl pridininium tetrafluoroborate[20] in stead of TrCl resulted in the rapid progress of the reaction and the high yield of the tritylated product. The tritylation of 3b followed by acetylation gave 2',3',4'-triacetyl-6'-trityl-10-deoxygeniposide (33) in 99.6 % yield. Detritylation of 33 with 80% acetic acid and subsequent oxidation with Jones reagent afforded 6'-acid triacetate (34). 6'-GABA amide (35), the hapten of type II, was derived from 34 via 6'-GABA amide methyl ester (36) according to the same procedure as mentioned above. Two haptens 30 and 35 were coupled with BSA to give immunogenic conjugates 28 and 31, respectively.

On the basis of the colorimetrical analysis for sugar moieties in glucosides by a phenol-sulfuric acid reagent,[21] the molar ratios of glucoside to BSA in six above-mentioned conjugates were as follows: 4.5 in 13, 8.5 in 16, 15.7 in 20, 15.2 in 25, 11.3 in 28 and 9.6 in 31.

Preparation and properties of antibodies by the six immunogenic conjugates described in the present paper as well as their application to the determination of three key intermediates, 1, 2 and 3 in various plants will be given elsewhere.

ACKNOWLEDGEMENT

We are grateful to Prof. F. Murai and Dr. M. Tagawa, Aichi Medical University, for their kind gift of boschnaloside and also to Prof. T. Shingu, Kobe-Gakuin University, for the measurement of 400 MHz NMR spectra.

EXPERIMENTAL

Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-435 infrared spectrometer and ultraviolet (UV) spectra on a Hitachi model 200-20 spectrometer. Optical rotations were obtained at 25℃ on JASCO DIP-181 and DIP-300 degital polarimeters. Proton and carbon nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were determined as solution in the indicated solvents with JEOL FX-200 which was used unless stated otherwise, Bruker AC-300 and AM-400 nuclear magnetic resonance spectorometers.

Merck silica gel GF_{254} or Merck 0.25 mm silica gel 60 F_{254} plates were used for thin layer chromatography (TLC) and Merck silica gel 60 PF_{254} for preparative thin layer chromatography (PLC). Column chromatography was carried out on M. Nagel silica gel 60 (70 - 200 mesh) or Wako

activated charcoal. Spots on TLC were visualized under UV light irradiation, by expodure to iodine vapor or by spraying with anisaldehyde - sulfuric acid followed by heating and bands on PLC under UV light irradiation. All nonaqueous reactions were carried out under nitrogen or argon. Anhydrous magnesium sulfate was used for drying organic solvent extracts.

6,6-Bis(ethylthio)-7-deoxyloganin tetraacetate (41)

Boron trifluoride etherate (2.0 ml) was added to an ice-cooled solution of 40 (2.317 g) and ethanedithiol (5.0 ml) in anhydrous tetrahydrofuran (THF) (7.0 ml). The mixture was stirred for 1 hr under ice-cooling and for an additional 2 days at room temperature. The reaction mixture was poured on iced water and extracted three times with $CHCl_3$. The extracts were washed successively with sat. aq. $NaHCO_3$ and water, and dried. Removal of the solvent gave a residue (8.235 g) which was chromatographed on silica gel with ether as eluant. The fractions showing Rf 0.48 on TLC (diethyl ether) were combined and concentrated in vacuo. The crystalline residue (2.617 g) was recrystallized from n-PrOH to yield colorless needles of 41.

mp 135 - 136 ℃. UV λ_{max} (MeOH) nm (log ε): 230(3.92). IR ν_{max} (KBr) cm^{-1}: 1758, 1717, 1636. ^{1}H-NMR ($CDCl_3$) δ : 7.28(1H, d, J=1.2 Hz, 3-H), 5.27(1H, d, J=5.1 Hz,1-H), 5.23(1H, t, J=9.3 Hz, 3'-H), 5.09(1H, t, J=9.5 Hz, 4'-H), 5.00(1H, dd, J=9.3 and 8.1 Hz, 2'-H), 4.85 (1H, d, J=8.1 Hz, 1'-H), 4.28(1H, dd, J=12.5 and 5.4 Hz, 6'-H), 4.13(1H, dd, J=12.2 and 2.4 Hz, 6'-H), 3.74(1H, ddd, J=9.5, 5.1 and 2.4 Hz, 5'-H), 3.71(3H, s, OCH_3), 3.53(1H, br. d, J=8.3 Hz, 5-H), 3.35 - 3.15(4H, m, $-SCH_2CH_2S-$), 2.48(1H, dd, J=11.7 and 5.4 Hz,7-H), 2.09, 2.03, 2.01 and 1.97 (each, 3H, s, 4 × $OCOCH_3$), 1.14(3H, d, J=6.1 Hz, 10-H_3). Anal. Calcd. for $C_{27}H_{36}O_{13}S_2$: C, 51.26; H, 5.74; S, 10.13. Found: C, 50.97 ; H, 5.69 ; S, 10.14.

7-Deoxyloganin tetraacetate (12)

A mixture of 41 (2.000 g) and Raney Ni (W-2) (20.0 g) in absolute EtOH (30 ml) was refluxed overnight. The catalyst was filtered off and washed with EtOH. The filtrate and washings were combined and concentrated in vacuo. The residue (1.746 g) was acetylated with acetic anhydride and pyridine (each 15 ml) in the usual way. The crude acetylated product (1.958 g) was chromatographed on silica gel (80 g) using diethyl ether as eluant. The combined fraction showing Rf 0.35 on TLC (diethyl ether) was concentrated in vacuo to give a crystalline residue (1.220 g) which was recrystallized from n-PrOH to yield colorless needles of 12.

mp 117-118.5 ℃. [α]$_D$ -80.83 ° (c, 0.98, MeOH). UV λ_{max} (MeOH) nm (log ε): 233(3.04). IR ν_{max} (KBr) cm^{-1}: 1758, 1742(sh),1715, 1640, 1630(sh). ^{1}H-NMR ($CDCl_3$) δ : 7.30(1H, d, J=1.2 Hz, 3-H), 5.23(1H, t, J=9.3 Hz, 3'-H), 5.16(1H, d, J=3.4 Hz, 1-H), 5.10(1H, t, J=9.3 Hz, 4'-H), 4.99(1H, dd,

J=9.0 and 8.1 Hz, 2'-H), 4.87 (1H, d, J=8.1 Hz, 1'-H), 4.31(1H, dd, J=12.5 and 4.6 Hz, 6'-H), 4.14(1H, dd, J=12.5 and 2.4 Hz, 6'-H), 3.74(1H, ddd, J=9.8, 4.6 and 2.4 Hz, 5'-H), 3.70(3H, s, OCH_3), 2.87 (1H, br. q, J=6.5 Hz, 5-H), 2.17(1H, m, 6-H), 2.09, 2.03, 2.00 and 1.93(each 3H, s, 4 × $OCOCH_3$), 1.90 - 1.76(2H, m, 8- and 9-H), 1.45(1H, d, J=15.0, 7.3 and 5.6 Hz, 6-H), 1.18(1H, m, 7-H), 1.06(3H, d, J=5.9 Hz, 10-H_3). ^{13}C-NMR ($CDCl_3$) δ : 170.54, 170.15, 169.38 and 169.11(each s, 4 × $COCH_3$), 167.45(s, 11-C), 149.45(d, 3-C), 113.23(s, 4-C), 96.04(d, 1-C), 95.38(d, 1'-C), 72.56(d, 3'-C), 72.12(d, 5'-C), 70.69(d, 2'-C), 68.35(d, 4'-C), 61.81(t, 6'-C), 51.11(q, 11-OCH_3), 48.26(d, 9-C), 34.96(d, 5-C), 33.01(t, 6-C), 32.64(d, 8-C), 31.16(t, 7-C), 20.70, 20.58 and 20.21(each q, 4 × $COCH_3$), 19.63(q, 10-C). Anal. Calcd. for $C_{25}H_{34}O_{13}$: C, 55.35; H, 6.32. Found: C, 55.34; H, 6.33.

7-Deoxyloganin (1b)

To a solution of 12 (228.3 mg) in anhydrous MeOH (4 ml) was added 0.26N NaOMe (0.2 ml) and the mixture was stirred for 2 hr at room temperature. After neutralization with Amberlite IR-120 resin (H^+ form), the resin was filtered off and washed with MeOH. The filtrate and washings were combined and concentrated in vacuo. The residue (154.3 mg) was purified by PLC (MeOH - $CHCl_3$ 2 : 8) and the band around Rf 0.35 afforded a crystalline residue (139.9 mg) which was recrystallized from n-PrOH to yield colorless needles of 1b.

mp 156 - 158℃. [α]$_D$ -92.24° (c, 0.98, MeOH) [lit. mp 157 - 157.5℃ from THF - hexane. [α]$_D$ -90° (c, 0.295, EtOH)].[22] UV λ_{max} (MeOH) nm(log ε): 236(4.06),IR ν_{max} (KBr) cm^{-1}: 3510, 3350, 1685, 1645. ^{1}H-NMR (CD_3OD) δ : 7.41(1H, d, J=1.2 Hz, 3-H), 5.21(1H, d, J=5.6 Hz, 1-H), 4.66(1H, d, J=7.8 Hz, 1'-H), 3.89(1H, dd, J=12.0 and 1.7 Hz, 6'-Ha), 3.69(1H, s, 11-OCH_3), 3.67(1H, dd, J=12.0 and 5.6 Hz, 6'-Hb), 3.38(1H, dd, J=9.0 and 7.8 Hz, 3'-H), 3.36 - 3.25(2H, m, 5'- and 4'-H), 3.19(1H, dd, J=8.8 and 7.8 Hz, 2'-H), 2.88(1H, br q, J=7.3 Hz, 5-H), 2.18(1H, dtdd, J=12.7, 7.6, 3.4 and 1.2 Hz, 6-Ha), 1.96(1H, br tq J=7.3 and 6.6 Hz, 8-H), 1.87(1H, dtd, J=11.5, 7.3 and 3.7 Hz, 7-H), 1.73(1-H, ddd, J=8.1, 6.1 and 5.6 Hz, 9-H), 1.38(1H, ddt, J=12.7, 9.3 and 7.3 Hz, 6-Hb), 1.18(1H, ddt, J=11.5, 9.3 and 3.7 Hz, 7-H), 1.08(3H, d, J=6.6 Hz, 10-H_3). ^{13}C-NMR (CD_3OD, 75.5MHz) δ : 169.75(s, 11-C), 152.70(d, 3-C), 112.97(s, 4-C), 100.25(d, 1'-C), 97.90(d, 1-C), 78.40(d, 3'-C), 78.10(d, 5'-C), 74.81(d, 2'-C),71.65(d, 4'-C), 62.82(t, 6'-C), 51.66(q, 11-OCH_3), 36.59(d, 5-C), 35.29(t, 6-C), 34.20(d, 8-C), 33.37(t, 7-C), 20.83(q, 10-C). Anal. Calcd. for $C_{17}H_{26}O_9$: C, 54.54; H, 7.00. Found: C, 54.51; H, 7.07.

7-Deoxyloganic acid (1a)

The compound 1b (500 mg) was hydrolyzed with 0.5N aq. NaOH (7.0 ml) for 5 hr under stirring at room temperature. The mixture was neutralized with Amberlite IR-120 resin (H^+ form) and the

resin was filtered off, then washed with water. The combined filtrate and washings were concentrated in vacuo to yield 1a as a colorless syrup.

$[\alpha]_D$ -89.38° (c 1.07, MeOH). ^{1}H-NMR (CD_3OD) δ : 7.42(1H, d, J=1.2 Hz, 3-H), 5.21(1H, d, J=5.9 Hz, 1-H), 4.68(1H, d, J=7.8 Hz, 1'-H), 3.90(1H, dd, J=11.7 and 1.5 Hz, 6'-H), 3.67(1H, t, J=8.8 Hz, 3'-H), 3.20(1H, dd, 8.8 and 7.8 Hz, 2'-H), 2.86(1H, tdd,J=8.0, 6.5 and 1.2 Hz, 5-H), 2.30 - 2.10(1H, m, 6-H), 2.07 - 1.85(2H, m, 7- and 8-H), 1.73(1H, ddd, J=8.3, 6.4 and 5.9 Hz, 9-H), 1.53 - 1.10(2H, m, 6- and 7-H), 1.09(3H, d, J=6.4 Hz 10-H_3). FAB-MS m/z: 361 $[M+H]^+$

7-Deoxyloganin 11-GABA amide methyl ester (14)

A solution of 1a (245.1 mg), 1-hydroxybenzotriazole (HOBT) (183.8 mg) and EDCI (156.6 mg) in anhydrous THF (2.0 ml) was stirred for 1 hr under ice- cooling. To the mixture was added a solution of methyl γ -aminobutylate hydrochloride (Me-GABA·HCl) (114.1 mg) and dimethylaminopyridine (DMAP) (166.9 mg) in anhydrous acetonitrile (1.0 ml) and the mixture was stirred for 1 hr at the same temperature. Stirring was continued for an additional 2 days at room temperature. Evaporation in vacuo of the solvent gave a residue which was purified by PLC (MeOH - $CHCl_3$ - HCOOH 30 : 65 : 5). The band around Rf 0.50 afforded 14 (298 mg) as a colorless syrup.

$[\alpha]_D$ -87.00° (c, 1.01, MeOH). IR ν_{max} (KBr) cm^{-1}: 3365.8, 2953.3, 2872.2, 1723.7, 1654.1, 1602.2, 1542.0. ^{1}H-NMR (CD_3OD) δ : 7.67(1H, br. t, J=5.6 Hz, NH), 7.05(1H, d, J=1.2 Hz, 3-H), 5.20(1H, d, J=5.1 Hz, 1-H), 4.66(1H, d, J=7.6 Hz, 1'-H), 3.89(1H, brd, J=12.7 Hz, 6'-H), 3.71 - 3.63(1H, m, 5'-H), 3.66(3H, s, OCH_3), 3.60(1H, dd, J=12.7 and 5.6 Hz, 6'-H), 3.39(1H, dd, J=9.0 and 8.8 Hz, 4'-H), 3.33 - 3.23(3H, m, 4"-H_2 and 3'-H), 3.21(1H, dd, J=8.6 and 7.8 Hz, 2'-H), 2.97(1H, br q, J=7.1 Hz, 5-H), 2.36(2H, t, J=7.3 Hz, 2"-H), 2.17 - 1.70(4H, m, 9-, 8-, 7- and 6-H), 1.81(2H, q, J=7.3 Hz, 3"-H), 1.34 - 1.04(2H, m, 7- and 6-H), 1.09(3H, d, J=6.6 Hz, 10-CH_3). ^{13}C-NMR (CD_3OD, 75.5MHz) δ : 175.48(s, 1"-C), 170.71(s, 11-C), 146.88(d, 3-C), 116.60(s, 4-C), 100.05(d, 1'-C), 97.13(d, 1-C), 78.36(d, 3'-C), 78.04(d, 5'-C), 74.84(d, 2'-C), 71.65(d, 4'-C), 62.80(t, 6'-C), 52.14(q, 11-OCH_3), 39.78(T, 6'-C), 36.33(d, 8-C), 34.87(d, 5-C), 34.05(t, 7-C), 32.53(t, 6-C), 32.20(t, 2"-C), 25.88(t, 3"-C), 20.70(q, 10-C). FAB-MS m/z: 460 $[M+H]^+$

7-Deoxyloganin 11-GABA amide (15)

A solution of 14 (217.2 mg) in MeOH (4.8 ml) was added to 0.4N aq. NaOH solution (4.8 ml) and the mixture stirred for 2 hr at room temperature. After neutralization with Amberlite IR-120 resin (H^+ form), the resin was filtered off and washed with MeOH. The filtrate and washings were combined and concentrated in vacuo. The residue was purified by PLC (MeOH - $CHCl_3$ 3 : 7) and

the product obtained from the band around Rf 0.2 was chromatographed on activated charcoal (400 mg). Elution with MeOH and afforded a crystalline residue which was recrystallized from n-PrOH to yield colorless needles of 15.

mp 216-218 ℃. UV λ_{max} (MeOH) nm (log ε): 228 (4.01). IR ν_{max} (KBr) cm^{-1}: 3501, 3425, 3250, 2963, 2915, 1711, 1649, 1613, 1528. ^{1}H-NMR (CD_3OD) δ : 7.05(1H, d, J=1.2 Hz, 3-H), 5.21(1H, d, J=5.1 Hz, 1-H), 4.66(1H, d, J=7.6 Hz, 1'-H), 3.89(1H, br. d, J=12.0 Hz, 6'-Ha), 3.68(1H,dd, J=12.0 and 5.4 Hz, 6'-Hb), 3.46 - 3.15(5H, m, 2'-, 3'-, 4'-H and 4"-H_2), 2.99(1H, br q, J=7.6 Hz, 5-H), 2.33(2H, t, J=7.3 Hz, 2"-H), 2.15 - 2.03(1H, m, 6-Ha), 2.02 - 1.70(3H, m, 8-, 7- and 9-H), 1.81(2H, quint, J=7.3 Hz, 3"-H_2), 1.45 - 1.13(2H, m, 6- and 7-H), 1.09(3H, d, J=6.4 Hz, 10-H3). FAB-MS m/z: 446 $[M+H]^+$

7-Deoxyloganin 11-GABA-BSA conjugate (13)

BSA (223.2 mg) was dissolved in water which was adjusted by the addition of 0.02N aq. NaOH. Upon addition of 15 (60.0 mg), lowering pH to 5.5 was observed, and EDCI (28.5 mg) was added subsequently. The mixture was stirred overnight at 25 ℃ and dialyzed at 4 ℃ against 10 × 5 l H_2O for 5 days. The inner solution was lyophilized to give 13 (230.1 mg). Coupling ratio was determined from the colorimetrical data by a phenol - sulfuric acid reagent for the sugar part of deoxyloganin in the conjugate 13 and the glucoside/protein ratio was calculated as 4.5.

6'-Carboxy-7-deoxyloganin (17)

The foregoing compound (1b) (258 mg) and Na_2CO_3 (430 mg) were added to the suspension of prehydrogenated PtO_2 (258 mg) in H_2O (2.5 ml) and the mixture was stirred for 7 days under an atmosphere of oxygen at room temperature. The reaction mixture was neutralized with Amberlite IR-120 resin (H^+ form) and the resin was filtered off, then washed with H_2O. The combined filtrate and washings were concentrated in vacuo to afford the residue (246.9 mg) which was subjected to PLC (MeOH - $CHCl_3$ - HCOOH 20 : 80 : 0.5). The residue obtained from the band around Rf 0.25 was chromatographed on activated charcoal (720 g). The MeOH eluate yielded 17 (129 mg) as a syrup.

IR ν_{max} (KBr) cm^{-1}: 3431, 2954, 1735, 1696, 1640, 1442. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ : 7.38(1H, d, J=1.13 Hz, 3-H), 5.19 - 5.11(1H, br s, COO*H*), 4.99(1H, d, J=6.1 Hz, 1-H), 4.60(1H, d, J=7.8 Hz, 1'-H), 3.68(1H, d, J=9.6 Hz, 5'-H), 3.35(1H, dd, J=9.5 and 9.1 Hz, 4'-H), 3.20(1H, t, J=8.9 Hz, 3'-H), 3.02(1H, dd, J=8.5 and 8.1 Hz, 2'-H), 2.79(1H, q, J=7.6 Hz, 5-H), 2.10(1H, ddd, J=12.6, 8.0 and 3.5 Hz, 6-H), 1.85(1H, sextet, J=6.7 Hz, 8-H), 1.76(1H, qd, J=7.6 and 3.5 Hz, 7-H), 1.66(1H, dt, J=8.1 and 6.1 Hz, 9-H), 1.26(1H, ddt, J=13.0, 8.8 and 7.8 Hz, 6-H), 1.20 - 1.15(1H, m, 7-H), 1.02(3H, d, J=6.5 Hz, 10-H). ^{13}C-NMR (DMSO-d_6, 75.5 MHz) δ : 169.95(s, 6'-C), 166.76(s, 11-C), 150.81(d, 3-C), 110.83(s,

4-C), 99.29(d, 1-C), 96.46(d, 1'-C), 75.76(d, 3'-C), 75.50(d, 5'-C), 72.61(d, 2'-C), 71.17(d, 4'-C), 50.82(q, 11-O$\underline{C}H_3$), 47.04(d, 9-C), 34.59(d, 5-C), 33.45(t, 6-C), 32.26(d, 8-C), 37.71(t, 7-C), 20.20(q, 10-C). FAB-MS m/z: 389 $[M+H]^+$

7-Deoxyloganin 6'-GABA amide methyl ester (18)

A solution of 17 (94.6 mg), HOBT (44.9 mg) and EDCI (51.4 mg) in anhydrous acetonitrile and anhydrous THF (1:1) (2.0 ml) was stirred for 1hr in a ice bath. To the mixture was added a solution of Me-GABA·HCl (44.9 mg) and DMAP (72.5 mg) in anhydrous acetonitrile (1.0 ml) and the mixture was stirred for 1 hr at the same temperature. After stirring the solution for an additional 2 days at room temperature. Evaporation of the solvent in vacuo gave a residue which was purified by PLC (MeOH - $CHCl_3$ - HCOOH 20 : 80 : 0.5). The band around Rf 0.60 afforded 18 (91.7 mg) as a colorless syrup.

IR ν_{max} (KBr) cm^{-1}: 3370, 2954, 1728, 1709, 1663, 1636, 1546. ^{1}H-NMR (CD_3OD) δ : 7.40(1H, d, J=1.0 Hz, 3-H), 5.14(1H, d, J=5.6 Hz, 1-H), 4.73(1H, d, J=7.8 Hz, 1'-H), 3.74(1H, d, J=9.5 Hz, 5'-H), 3.69(3H, s, 11- OC$\underline{H}_3$), 3.65(3H, s, 4"-OC$\underline{H}_3$), 3.53(1H, dd, J=9.3 and 8.5 Hz, 4'-H), 3.42(1H, dd, J= 8.8 and 8.5 Hz, 3'-H), 3.35 - 3.20(3H, m, 2'- and 4"-H_2), 2.89(1H, q, J=7.6 Hz, 5-H), 2.37(3H, t, J=7.3 Hz, 2"-H), 2.19(1H, br. dtd, J=12.2, 7.6 and 3.4 Hz, 6-H), 1.96(1H,br.tq, J=7.3 and 6.4 Hz, 8-H), 1.82(3H, quint. J=7.3 Hz, 3"-H), 1.76(1H, ddd, J=8.3, 6.11 and 5.9 Hz, 9-H), 1.37(1H, br.ddt, J=12.5, 7.6 and 3.4 Hz, 7-H), 1.19(1H, ddt, J=12.9, 7.6 and 3.4 Hz, 7-H), 1.08(3H, d, J=6.4 Hz, 10-H_3). ^{13}C-NMR (CD_3OD) δ : 175.36(s, 4"-C), 171.44(s, 6'-C), 169.62(s, 11-C), 152.49(d, 3- C), 113.09(s, 4-C), 100.56(d, 1'-C), 98.18(d, 1-C), 77.55(d, 3'-C), 76.94(d, 5'-C), 74.26(d, 2"-C), 73.12(d, 4'-C), 52.13(q, 1"-O$\underline{C}H_3$),51.69(q, 11-O$\underline{C}H_3$), 49.21(d, 9-C), 39.45(t, 4"-C), 36.63(d, 8-C), 35.22(d, 5-C), 34.13(t, 7-C), 33.40(t, 6-C), 32.01(t, 2"-C), 25.59(t, 3"-C), 20.82(q, 10-C). Anal. Calcd. for $C_{22}H_{38}O_{11}N\cdot 1/2H_2O$: C, 53.22; H, 6.90; N, 2.82. Found: C,53.10 ; H, 6.87; N, 2.83. FAB-MS m/z: 488 $[M+H]^+$.

7-Deoxyloganin 6'-GABA amide (19)

Compound 18 (159.2 mg) was hydrolyzed in MeOH (4.0 ml) with 0.4N aq. NaOH (4.0 ml) for 1 hr by stirring at room temperature. The mixture was worked up as described above for the preparation of 15. The crude products were chromatographed on activated charcoal (560 mg). Elution with MeOH afforded 19 (123 mg) as a white amorphous foam.

IR ν_{max} (KBr) cm^{-1}: 3450, 3410, 2920, 2850, 1625, 1573, 1528. ^{1}H-NMR (CD_3OD) δ : 7.40(1H, d, J=1.2 Hz, 3'-H), 5.14(1H, d, J=5.9 Hz, 1-H), 4.73(1H, d, J=7.8 Hz, 1'-H), 3.74(1H, d, J=9.5 Hz, 5'-H), 3.69(3H, s, 11-OC$\underline{H}_3$), 3.54(1H, dd, J=9.8 and 8.6 Hz, 4'-H), 3.42(1H, t, J=8.8 Hz, 3'-H),3.36 - 3.18(3H,

m, 2'- and 4"- H), 2.89(1H, br. q, J=7.8 Hz, 5-H), 2.34(2H, t, J=7.3 Hz, 2"-H), 2.28 - 2.10(1H, m, 6-Ha), 2.05 - 1.70(3H, m, 8-, 7- and 9-H), 1.81(2H, quintet, 3"-H), 1.46 -1.12(2H, m, 6- and 7-H), 1.08(3H, s, 10-H_3). FAB-MS m/z: 474 $[M+H]^+$.

7-Deoxyloganin 6'-GABA-BSA conjugate (16)

BSA (222.7 mg) was dissolved in water which was adjusted to pH 8.5 by the addition of 0.02N aq. NaOH. 19 (63.9 mg) and EDCI (28.5 mg) were added subsequently. The mixture was stirred overnight at 25 ℃ and dialyzed at 4 ℃ against 10 × 5 l changes of H_2O for 5 days. The inner solution was lyophilized to give the conjugate 16 (236.3 mg). The conjugate 16 was determined from the colorimetrical method by phenol - sulfuric acid for the sugar moiety to contain 8.5 mole of 19 / mole of BSA.

7-Deoxy-8-epi-loganic acid tetraacetate (23)

The title compound was prepared according to the procedure of S. P. Bal et al.[15] To a solution of boschnaloside tetraacetate (22) (1.000 g) in t-BuOH (41.1 ml) was added 2-methyl-2-butene (9.9 ml) and subsequently dropwise a solution of sodium chlorite (1.641 g) and sodium dihydrogen phosphate (2.133 g) in water (16.5 ml) over a 15 min period by stirring at room temperature. Stirring was continued for 40 hr at the same conditions. The reaction mixture was concentrated in vacuo and the residue was partitioned between water and $CHCl_3$. The aqueous layer was further extracted with $CHCl_3$. The extracts were washed with sat. brine, dried and concentrated in vacuo. The residue (1.020 g) was chromatographed on silica gel (30 g) using $CHCl_3$-MeOH with the increasing ratio of MeOH. The 5% MeOH-$CHCl_3$ eluates were combined and concentrated in vacuo to give a crystalline residue (912.4 mg) which was recrystallized from aqueous EtOH to yield colorless needles of 23.

mp 188 - 189.5 ℃. $[\alpha]_D$ -98.89 ° (c, 1.00, MeOH). IR ν_{max} (KBr) cm^{-1}: 3450, 2950, 1758, 1720, 1646. ^{1}H-NMR ($CDCl_3$) δ : 7.45(1H, br s, 3-H), 5.21(1H, br t, J=9.3 Hz, 3'-H), 5.10(1H, d, J=4.0 Hz, 1-H), 5.09(1H, br t, J=9.4 Hz, 4'-H), 5.06(1H, dd, J=9.0 and 8.1 Hz, 2'-H), 4.85(1H, d, J=8.1 Hz, 1'-H), 4.27(1H, dd, J=12.2 and 5.1 Hz, 6'-H), 4.14(1H, dd, J=12.2 and 2.4 Hz, 6'-H), 3.77(1H, ddd, J=9.8, 5.1 and 2.4 Hz, 5'-H), 2.90(1H, br q, J=7.1 Hz, 5-H), 2.40 - 2.10(2H, m, 8- and 9-H), 2.09, 2.04, 2.03 and 2.01(each 3H, s, 4 × OCOCH_3), 1.90 - 1.70(1H, m, 7-H), 1.55(1H, dtd, J=11.5, 6.6 and 4.9Hz, 6-H), 1.29(1H, dq, J=12.5 and 8.1 Hz, 7-H), 1.02(3H, d, J=6.6 Hz, 10-H_3). ^{13}C-NMR ($CDCl_3$) δ :172.51(s, 11-C), 170.66, 170.35, 169.38, 169.18(each s, 4 × $COOCH_3$), 152.86(d, 3-C), 111.46(s, 4-C), 99.73(d, 1'-C), 99.49(d, 1-C), 73.12(d, 3'-C), 72.29(d, 5'-C), 71.56(d, 2'-C), 68.26(d, 4'-C), 62.13(t, 6'-C), 42.93(d, 9-C), 36.20(d, 5-C), 33.33(d, 8-C), 32.18(t, 6-C), 31.16(t, 7-C), 20.72(q, 2 × $COCH_3$),

20.60(q, 2 × CO<u>C</u>H$_3$), 16.37(q, 10-C). Anal. Calcd. for $C_{24}H_{32}O_{13}$: C, 54.54; H, 6.10. Found: C, 54.67; H, 6.18.

7-Deoxy-8-epi-loganin 11-GABA amide (<u>24</u>)

A solution of <u>23</u> (100 mg), HOBT (51.2 mg) and EDCI (39.9 mg) in anhydrous THF (1.5 ml) was stirred for 1 hr in a ice bath. To the solution was added a solution of Me-GABA·HCl (31.7 mg) and DMAP (46.4 mg) in anhydrous acetonitrile (1.0 ml) and the mixture was stirred for 1 hr at the same temperature. Stirring was continued for an additional 1 day at room temperature. The reaction mixture was diluted with water and extracted with $CHCl_3$. The extracts were washed, successively, with 1N HCl, sat. aq. $NaHCO_3$ and water, and dried. Removal of the solvent gave a residue which was purified by PLC ($CHCl_3$ - MeOH 95 : 5). The band around Rf 0.60 afforded a crystalline residue (112.0 mg) which was recrysallized from i-PrOH to give tetraacetyl 7-deoxy-8-epi-loganin 11-GABA amide methyl ester as colorless needles.

mp 139 - 140 ℃. UV λ_{max} (MeOH) nm (log ε): 227.7 (4.02). IR ν_{max} (KBr) cm^{-1}: 3400, 2950, 1750, 1659, 1622. ^{1}H-NMR (CD_3OD, 300 MHz) δ : 7.63(1H, br t, J=5.5 Hz, N<u>H</u>), 7.10(1H, d, J=1.0 Hz, 3-H), 5.31(1H, d, J=2.9 Hz, 1-H), 5.28(1H, d, J=9.5 Hz, 4'-H), 5.04(1H, d, J=8.0 Hz, 2'-H), 5.01(1H, d, J=9.5 Hz, 1'-H), 4.87(1H, dd, J=9.7 and 8.1 Hz, 4'-H), 4.29(1H, dd, J=12.4 and 4.3 Hz, 6'-H), 4.17(1H, dd, J= 12.4 and 2.4 Hz, 6'-H), 3.92(1H, ddd, J=10.0, 4.3 and 2.4 Hz, 5'-H), 3.66(3H, s, OC<u>H</u>$_3$), 3.21(2H, quint, J=6.8, 4"-H), 2.94(1H, td, J=7.7 and 4.1 Hz, 5-H), 2.36(2H, t, J=7.4 Hz, 2"-H), 2.39 - 2.22(2H, m, 8- and 9-H), 2.06, 2.01, 1.96 and 1.92(each 3H, s, OCOC<u>H</u>$_3$), 1.82(2H, quint, J=7.1 Hz, 2"-H), 1.99 - 1.73(2H, m, 6- and 7-H), 1.51(1H, ddd, J=11.7, 7.6 and 4.1 Hz, 6-H), 1.28(1H, dq, J=12.3 and 8.1, 7-H), 1.03(3H, d, J=6.8 Hz, 10-H). ^{13}C-NMR (CD_3OD) δ : 173.90(s, 1"-C), 170.54 and 170.06(each s, O<u>C</u>OCH$_3$), 169.40(s, 2 × O<u>C</u>OCH$_3$), 166.70(s, 11-C), 146.27(d, 3-C), 115.47(s, 4-C), 95.84(d, 1'-C), 94.36(d, 1-C), 72.63(d, 3'-C), 72.12(d, 5'-C), 70.79(d, 2'-C), 68.47(d, 4'-C), 61.83(t, 6'-C), 51.74(q, 1"-O<u>C</u>H$_3$), 43.01(d, 9-C), 39.07(t, 4"-C), 35.15(d, 5-C), 33.13(t, 6-C), 31.89(d, 8-C), 31.65(t, 2"-C), 30.94(t, 7-C), 24.67(t, 3"-C), 20.70(q, OCO<u>C</u>H$_3$), 20.57(q, 2 × OCO<u>C</u>H$_3$), 20.41(q, OCO<u>C</u>H$_3$), 15.96(q, 10-C). Anal. Calcd. for $C_{29}H_{41}O_{14}N$: C, 55.50; H, 6.58; N, 2.23. Found: C, 55.53; H, 6.51; N, 2.10.

The above amide methyl ester (236.8 mg) was hydrolyzed in MeOH (4.7 ml) with 0.4N aq. NaOH (4.7 ml) for 1.5 hr by stirring at room temperature. The mixture was worked up as described above for the preparation of <u>15</u>. The crude products were purified by PLC (Rf 0.10, CHCl3 - MeOH - HCOOH 20 : 80 : 5) followed by column chromatography on activated charcoal (800 mg) with MeOH to afford <u>24</u> (179.4 mg) as a white amorphous foam.

IR ν_{max} (KBr) cm^{-1}: 3360, 2920, 2860, 1710, 1655, 1595. ^{1}H-NMR (CD_3OD) δ : 7.08(1H, d, J=1.0 Hz, 3-H), 5.42(1H, d, J=3.9 Hz, 1-H), 4.67(1H, d, J=7.6 Hz, 3-H), 3.90(1H, dd, J=11.7 and 1.7 Hz, 6'-H),

3.71(1H,dd, J=11.7 and 5.4 Hz, 6'-H), 3.25(2H, t, J=6.6 Hz, 4"-H_2), 3.20(1H, dd, J=9.0 and 7.8 Hz, 2'-H), 2.97(1H, br td like Hz, 5-H), 2.33(2H, t, J=7.3 Hz, 2"-H_2), 2.16 - 2.35(2H, m, 8-H and 9-H), 1.99(1H, dq, J=11.7 and 8.1 Hz, 6-H), 1.90 - 1.61(1H, m, 7-H), 1.81(2H, quint, J=7.1Hz, 3"-H_2), 1.61 - 1.23(2H, m, 6-H and 7-H), 1.08(3H, d, J=6.6 Hz, 10-H_3). Anal. Calcd. for $C_{20}H_{31}O_{10}N$: C, 53.93; H, 7.01; N, 3.14. Found: C, 53.63; H, 6.98; N, 3.16. FAB-MS m/z: 446 $[M+H]^+$

7-Deoxy-8-epi-loganin 11-GABA-BSA conjugate (20)

BSA (443.0 mg) and 24 (179.4 mg) were condensed using EDCI (66.2 mg) according to the same procedure as described for 13. Dialysis followed by lyophilization provided 20 (489.9 mg). The 24 /BSA ratio of the conjugate 20 was determined by the aforementioned colorimetric method to give 15.7 mole of 24 / mole of BSA.

7-Deoxy-8-epi-loganin (2b)

Diazomethane in diethylether was added to a solution of 23 (100.2 mg) in MeOH (5 ml) in an ice bath until persistent yellow color. After leaving on an ice bath for 30 min, the mixture was concentrated in vacuo. The residue was purified by PLC (Rf 0.60, acetone - benzene 20 : 80) to give a crystalline residue (95.8 mg) which was recrystallized from i-PrOH to yield tetraacetyl 7-deoxy-8-epi-loganin as colorless needles.

mp 111 - 112 ℃. $[\alpha]_D$ -111.12 ° (c, 0.71, MeOH) [lit. mp 150 - 152℃. from AcOEt. $[\alpha]_D$ -128 ° (c, 0.4, MeOH)].[23] UV λ_{max} (MeOH) nm (log ε): 237.1(4.08). IR ν_{max} (KBr) cm^{-1}: 2950, 1758, 1705, 1640. ^{1}H-NMR ($CDCl_3$, 400MHz) δ : 7.35(1H, d, J=1.0 Hz, 3-H), 5.27(1H, d, J=3.5 Hz,1-H), 5.22(1H, t, J=9.6 Hz, 3'-H), 5.10(1H, dd, J=9.8 and 9.5 Hz, 4'-H), 4.99(1H, dd, J=9.6 and 8.1 Hz, 2'-H), 4.87(1H, d, J=8.1 Hz, 1'-H), 4.27(1H, dd, J=12.4 and 4.5 Hz, 6'-H), 4.17(1H, dd, J=12.4 and 2.4 Hz, 6'-H),3.73(1H, ddd, J=10.0, 4.4 and 2.5 Hz, 5'-H), 3.70(3H, s, $COOCH_3$),2.87(1H,tdd, J=8.0, 5.0 and 1.0 Hz, 5-H), 2.31 (1H, td, J=8.6 and 3.6 Hz, 9-H), 2.24(1H, tdq, J=8.6, 7.2 and 7.0 Hz, 8-H), 2.09, 2.03, 2.00 and 1.94 (each 3H, s, 4 × $COOCH_3$), 1.75(1H, dtd, J=12.3, 7.3 and 4.4 Hz, 7-H), 1.60(1-H, ddt, J=12.6, 7.6 and 4.9 Hz, 6-H), 1.24(1H, dq, J=12.6 and 8.1 Hz, 7-H), 1.00(3H, d, J=7.0 Hz, 10-H_3). ^{13}C-NMR ($CDCl_3$) δ : 170.57, 170.18, 169.38, and 169.16(each s, 4 × $COCH_3$), 167.36(s, 11-C), 150.21(d, 3-C), 113.26(s, 4-C), 95.69(d, 1'-C), 94.70(d,1-C), 72.61(d, 3'-C), 72.08(d, 5'-C), 70.74(d, 2'-C), 68.35(d, 4'-C), 61.71(t, 6'-C), 51.11(q,OCH_3), 42.67(d, 9-C), 35.44(d, 5-C), 32.38(d, 8-C), 31.09(t, 7-C), 20.70(q, $COCH_3$), 20.58(q, 2 × $COCH_3$), 20.26(q, $COCH_3$), 16.05(q, 10-C). Anal. Calcd. for $C_{25}H_{34}O_{13}$:C, 55.35; H, 6.32. Found: C, 55.35; H, 6.34.

To a solution of the above acetate (450 mg) in anhydrous MeOH (9 ml) was added 0.26N NaOMe (0.2 ml) and the mixture was stirred for 4 hr at room temperature. Work up in the same manner as

described for 1b gave 2b (301.0 mg) as colorless needles which was recrystallized from n-PrOH. mp 163-165℃. [α]$_D$ -115.13° (c, 0.72, MeOH). UV λ_{max} (MeOH) nm (log ε): 237.1(4.08). IR ν_{max} (KBr) cm^{-1}: 3750, 2960, 2900, 1682, 1642. ^{1}H-NMR (CD_3OD) δ : 7.42(1H, d, J=1.0 Hz, 3-H), 5.46(1H, d, J=4.6 Hz, 1-H), 4.68(1H, d, J=7.6 Hz, 1'-H), 3.90(1H, dd, J=11.7 and 1.7 Hz, 6'-Ha), 3.68(3H, s, 11-OCH_3), 3.64(1H, dd, J=11.72 and 5.62 Hz, 6'-Hb), 3.37(1H, t, J=8.6 Hz, 4'-H), 3.27(1H, dd, J=8.1 and 7.8 Hz, 3'-H), 3.19(1H, dd, J=9.0 and 7.8 Hz, 2'-H), 2.92(1H, td, J=7.8 and 6.1 Hz, 5-H), 2.40 - 2.15(2H, m, 9- and 8-H), 2.06(1H, dq, J=12.7 and 7.8 Hz, 6-Ha), 1.86 - 1.70(1H, m, 7-Ha), 1.63 - 1.46 (1H, m, 6-Hb), 1.39(1H, dq, J=12.2 and 7.8 Hz, 7-Hb), 1.08(3H, d, J=6.6 Hz, 10-CH_3). ^{13}C-NMR (CD_3OD) δ : 169.59(s, 11-C), 152.81(d, 3-C), 113.38(s, 4-C), 99.81(d, 1'-C), 96.25(d, 1-C), 78.42(d, 3'-C), 78.06 (d, 5'-C), 74.85(d, 2'-C), 71.81(d, 4'-C), 63.00(t, 6'-C), 51.64(q, 11-OCH_3), 44.44(d, 9-C), 37.58(d, 8-C), 34.59(d, 5-C), 33.28(t, 7-C), 32.38(t, 6-C), 16.76(q, 10-C). Anal. Calcd. for $C_{17}H_{26}O_9$:C, 54.54; H, 7.00. Found: C, 54.70; H, 7.07.

6'-Carboxy-7-deoxy-8-epi-loganin (27)

The compound 2b (240 mg) and Na_2CO_3 (524.8 mg) were added to the suspension of prehydrogenated PtO_2 (240 mg) in water (2.4 ml) and the mixture was stirred under an atmosphere of oxygen for 6 days at room temperature. The same work up procedure as described for 17 afforded a residue (212.5 mg) which was subjected to PLC (MeOH - $CHCl_3$ - HCOOH 20 : 80 : 0.5). The product obtained from the band around Rf 0.30 was chromatographed on activated charcoal (800 mg). The MeOH eluate yielded a crystalline residue (186.5 mg) which was recrystallized from i-PrOH to afford colorless plates of 27.

mp 111-118 ℃. IR ν_{max} (KBr) cm^{-1}: 3420, 2951, 1734, 1697, 1684, 1648, 1636. ^{1}H-NMR (CD_3OD, 300MHz) δ : 7.42(1H, d, J=0.9 Hz, 3-H), 5.34(1H, d, J=4.7 Hz, 1-H), 4.75(1H, d, J=7.8 Hz, 1'-H), 3.83(1H, d, J=8.8 Hz, 5'-H), 3.69(3H, s, OCH_3), 3.56(1H, t, J=9.0 Hz, 4'-H), 3.41(1H, t, J=8.9 Hz, 3'-H), 3.26(1H, dd, J=9.0 and 7.9 Hz, 2'-H), 2.92(1H, td, J=7.8 and 6.2 Hz, 5-H), 2.37 - 2.20(2H, m, 8- and 9-H), 2.07(1H, dq, J=13.1 and 8.4 Hz, 6-H), 1.79(1H, dtd, J=13.1, 8.6 and 5.6 Hz, 6-H), 1.32(1H, dq, J=12.4 and 8.3 Hz, 7-H), 1.08(3H, d, J=6.7 Hz, 10-H). ^{13}-CNMR (CD_3OD, 75.5MHz) δ : 172.73(s, 6'-C), 169.54(s, 11-C), 152.64(d, 3-C), 113.48(s, 4-C), 100.13(d, 1'-C), 96.45(d, 1-C), 77.42(d, 3'-C), 76.79(d, 5'-C), 74.46(d, 2'-C), 73.04(d, 4'-C), 51.67(q, 11-OCH_3), 44.30(d, 9-C), 37.59(d, 8-C), 34.60(d, 5-C), 33.19(t, 7-C), 32.35(t, 6-C), 16.65(q, 10-C). Anal. Calcd. for $C_{17}H_{24}O_{10} \cdot 1/2H_2O$: C, 51.38; H, 6.34. Found: C, 51.14; H, 6.54.

7-Deoxy-8-epi-loganin 6'-GABA amide (26)

A solution of 27 (100 mg), HOBT (43.1 mg) and EDCI (54.3 mg) in a mixture of anhydrous

acetonitrile and anhydrous THF (1:1) (2.0 ml) was stirred for 1hr under ice-cooling. To the mixture was added a solution of Me-GABA·HCl (43.1 mg) and DMAP (69.2 mg) in anhydrous acetonitrile (1.0 ml) and the mixture was stirred for 1 hr at the same temperature. Stirring was continued for an additional 2 days at room temperature. Evaporation in vacuo of the solvent gave a residue which was purified by PLC (MeOH - $CHCl_3$ - HCOOH 20 : 80 : 0.5). The band around Rf 0.60 afforded a crystalline residue (109.7 mg) which was recrystallized from $CHCl_3$ - petr. ether to afford colorless needles of 7-deoxy-8-epi-loganin 6'-GABA amide methyl ester.

mp 152-153 ℃. UV λ_{max} (MeOH) nm (log ε): 237.6(4.10). IR ν_{max} (KBr) cm^{-1}: 3420, 3340, 2950, 1735, 1714, 1700, 1642. ^{1}H-NMR (CD_3OD) δ : 7.41(1H, d, J=1.0 Hz, 3-H),5.39(1H, d, J=4.6 Hz, 1-H), 4.74(1H, d, J=7.8 Hz, 1'-H),3.72(1H, d, J=9.5 Hz, 5'-H), 3.69(3H, s, 11-COOC$\underline{H}_3$),3.65(3H, s, 1"-OC$\underline{H}_3$), 3.53(1H, dd, J=9.5 and 9.0 Hz, 4'-H), 3.40(1H, dd, J=9.0 and 8.5 Hz, 3'-H), 3.29(2H, t, J=7.1 Hz, 4"-H), 3.25(1H, dd, J=8.5 and 7.8 Hz, 2'-H), 2.92(1H, br td, J=14.0 and 7.6 Hz, 5-H), 2.37(2H, t, J=7.3 Hz, 2"-H), 2.37 - 2.20(2H, m, 8- and 9-H), 2.07(1H, m, 6-H), 1.82(2H, quintet, J=7.3 Hz, 3"-H), 1.96 - 1.61(1H, m, 7-H), 1.54 (1H, dddd, J=12.7, 8.3, 5.9 and 4.6 Hz, 6-H), 1.32(1H, dtd, J=11.7, 8.3 and 7.3 Hz, 7-H), 1.08(3H, d, J=6.8 Hz, 10-H_3). ^{13}C-NMR (CD_3OD) δ : 175.41(s, 1"-C), 171.54(s, 6'-C), 169.52(s, 11-C), 152.59(d, 3-C), 113.62(s, 4-C), 100.15(s, 1'-C), 96.52(d,1-C), 77.57(d, 5'-C), 76.75(d, 3'-C), 74.36(d, 2'-C), 73.17(d, 4'-C), 52.15(q, 4"-O$\underline{C}H_3$), 51.67(q, 11-O$\underline{C}H_3$), 44.39(d, 5-C), 39.50(t, 4"-C), 37.56(d, 9-C), 34.59(d,8-C), 33.37(t, 6-C), 32.38(t, 7-C), 32.08(t, 2"-C), 25.64(t, 3"-C), 16.81(q, 10-C). Anal. Calcd. for $C_{22}H_{33}O_{11}N$: C, 54.20; H, 6.82; N, 2.87. Found: C, 54.33; H, 6.70; N, 2.99.

The foregoing amide methyl ester (200.0 mg) was hydrolyzed with 0.4N aq. NaOH (4.0 ml) in MeOH (4.0 ml) for 20 min under stirring at room temperature. The mixture was worked up as described above for the preparation of 15. The crude products were chromatographed on activated charcoal (800 mg). Elution with MeOH afforded 26 (175 mg) as a white amorphous foam.

UV λ_{max} (MeOH) nm (log ε): 236.5(4.01). IR ν_{max} (KBr) cm^{-1}: 3450, 3300, 2950, 1706, 1666, 1645. ^{1}H-NMR (CD_3OD) δ : 7.41(1H, d, J=1.0 Hz, 3-H), 5.39(1H, d, J=4.6 Hz, 1-H), 4.74(1H, d, J=7.6 Hz, 1'-H), 3.73(1H, dd, J=9.3 Hz, 5'-H), 3.69(3H, s, 11-OC$\underline{H}_3$), 3.53(1H, dd, J=9.3 and 8.6 Hz, 4'-H), 3.40(1H, dd, J=9.0 and 8.6 Hz, 3'-H), 3.26(2H, t, J=7.3 Hz, 4"-H_2), 2.92(1H, td, J=7.8 and 6.1 Hz, 5-H), 2.34(2H, t, J=7.3 Hz, 2"-H_2), 2.38 - 2.22(2H, m, 9- and 8-H), 2.07(1H, dtd, J=12.7, 8.3 and 8.1 Hz, 6-Ha), 1.81(2H, quintet, J=7.3 Hz, 3"-H_2), 1.85 - 1.71(1H, m, 7-Ha), 1.54(1H, dddd, 12.7, 8.3, 6.1 and 4.9 Hz, 6-Hb), 1.32(1H, dq, J=12.0 and 8.1 Hz, 7-Hb), 1.08(3H, d, J=6.8 Hz, 10-H_3). Anal. Calcd. for $C_{21}H_{31}O_{11}N$: C, 53.27; H, 6.60; N, 2.96. Found: C, 53.02; H, 6.67; N, 2.95. FAB-MS m/z: 474 $[M+H]^+$

7-Deoxy-8-epi-loganin 6'-GABA-BSA conjugate (25)

BSA (450 mg) and 26 (140 mg) were condensed using EDCI (66.2 mg) acccording to the same procedure as described for 13. Dialysis followed by lyophilization provided 25 (496.1 mg). The *26 /BSA ratio of the conjugate 25 was determined by the aforementioned colorimetric method to* give 15.2 mole of 26 / mole of BSA.

10-Deoxygeniposide tetraacetate (29)

Geniposide (37) (327.9 mg) was hydrogenolyzed in MeOH (8 ml) with 5% Pd – C (50 mg) in the presence of 60% $HClO_4$ (6.5 μ l) under an atmosphere of hydrogen. After the uptake of 0.85 equivalent (16 ml) of hydrogen, the reaction was ceased. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo to give a residue (312.3 mg) which was acetylated with acetic anhydride (1 ml) and pyridine (2 ml) overnight at 4 ℃. The reaction mixture was poured on iced water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed successively with 1N HCl, sat. aq. $NaHCO_3$ and water, dried and concentrated in vacuo to give a residue which was purified by PLC (diethyl ether). The band around Rf 0.65 gave a crystalline product which was recrystallized from i-PrOH to yield colorless needles of 29.

mp 116 – 118 ℃. $[\alpha]_D$ -19.83° (c, 1.01, MeOH). UV λ_{max} (MeOH) nm (log ε): 236(3.98). IR ν_{max} (KBr) cm^{-1}: 1756, 1707, 1636. ^{1}H-NMR ($CDCl_3$) δ : 7.39(1H, d, J=1.0 Hz, 3-H), 5.45(1H, quint, J=1.7 Hz, 7-H), 5.18(1H, t, J=9.3 Hz, 3'-H), 5.11(1H, t, J=9.5 or 9.0 Hz, 4'-H), 5.02(1H, t, J=9.3, 7.8 Hz, 2'-H), 4.88(1H, d, J=8.1 Hz, 1'-H), 4.30(1H, dd, J=12.2 and 4.4 Hz, 6'-H), 4.14(1H, dd, J=12.2 and 2.4 Hz, 6'-H), 3.73(1H, ddd, J=10.0, 4.4 and 2.4 Hz, 5'-H), 3.70(1H, s, 11-COOC$\underline{H}_3$), 3.14(1H,tdd, J=8.1, 4.6 and 1.0 Hz, 5-H), 2.84 – 2.64(2H, m, 9- and 6-H), 2.19 – 2.13(1H, m, 6-H), 2.08, 2.03, 2.01 and 1.97(each 3H, s, 4 × OCOC$\underline{H}$3), 1.77(3H, br s, 10-$\underline{H}_3$). ^{13}C-NMR ($CDCl_3$) δ : 170.54, 170.18, 169.38 and 169.18(each s, O$\underline{C}$OCH$_3$), 167.48(s, 11-$\underline{C}$OOMe), 150.74(d, 3-C), 137.58(s, 4-C), 127.10(d, 7-C), 112.70(d, 8-C), 96.42(d, 1'-C), 98.48(d, 1-C), 72.61(d, 5'-C), 72.10(d, 3'-C), 70.79(d, 2'-C), 68.35(d, 4'-C), 61.76(t, 6'-C), 51.16(q, 11-O$\underline{C}$H$_3$), 49.19(d, 9-C), 38.41(t, 6-C), 33.28(d, 5-C), 20.70(q, CO$\underline{C}$H$_3$), 20.60(q, 2 × CO$\underline{C}$H$_3$), 20.34(q, CO$\underline{C}$H$_3$), 15.32(q, 10-C). Anal. Calcd. for $C_{25}H_{32}O_{13}$: C, 55.55; H, 5.97. Found: C, 55.29; H, 5.94.

10-Deoxygeniposide (3b)

The acetate (29) (2.000 g) was deacetylated in absolute MeOH (20 ml) with 0.26N NaOMe (0.7 ml) for 4 hr under stirring at room temperature. The same work up as described above for 1b afforded a crystalline product (1.363 g) which was recrystallized from i-PrOH to yield colorless needles of 3b.

mp 169 - 171 ℃. [α]$_D$ -5.50° (c, 1.00, MeOH). UV λ_{max} (MeOH) nm (log ε): 238(3.91). IR ν_{max} (KBr) cm^{-1}: 3540, 3420, 3380, 3250(sh), 1718, 1640. ^{1}H-NMR (CD_3OD) δ : 7.47(1H, d, J=1.2 Hz, 3-H), 5.47(1H, m, J=1.2 Hz, 7-H), 5.27(1H, d, J=6.6 Hz, 1-H), 4.71(1H, d, J=7.8 Hz, 1'-H), 3.88(1H, dd, J=12.0 and 1.7 Hz, 6'-Ha), 3.64(1H, dd, J=11.7 and 5.4 Hz, 6'-Hb), 3.39(1H, dd, J=9.0 and 8.8 Hz, 3'-H), 3.25(1H, dd like, J=9.0 and 8.8 Hz, 4'-H), 3.21(1H, dd, J=8.8 and 7.8 Hz, 2'-H), 3.12(1H, tdd, J=7.8, 6.6 and 1.2 Hz, 5-H), 2.72(1-H, br dd, J=15.9 and 7.8 Hz, 6-Ha), 2.63(1H, br dd like, J=7.8 and 6.6 Hz, 9-H), 2.06 (1H, ddq, J=15.9, 6.6 and 2.2 Hz, 6-Hb), 1.81(1H, br s, 10-H_3). ^{13}C-NMR (CD_3OD) δ : 169.71(s, 11-C), 153.25(d, 3-C), 140.14(s, 8-C), 127.78(d, 7-C), 112.84(s, 4-C), 100.00(d, 1'-C), 97.54(d, 1-C), 78.35(d,5'-C), 77.94(d, 3'-C), 74.82(d, 2'-C), 71.59(d, 4'-C), 62.76(t, 6'-C), 51.74(q, 11-O<u>C</u>H_3), 50.28(d, 9-C), 39.70(t, 6-C), 35.71(d, 5-C), 16.22(q, 10-C). Anal. Calcd. for $C_{17}H_{24}O_9$: C, 54.83; H, 6.50. Found: C, 54.53; H, 6.60.

10-Deoxygeniposidic acid (<u>3a</u>)

The compound <u>3b</u> (1.817 g) was hydrolyzed with 0.5N NaOH (25 ml) under stirring overnight at room temperature. Workup as described above for <u>1a</u> afforded colorless needles of <u>3a</u> (1.538 g) which were recrystallized from n-PrOH.

mp 115.5-117 ℃. [α]$_D$ -3.84° (c, 0.99, MeOH). IR ν_{max} (KBr) cm^{-1}: 3400, 2960, 2910, 2850(sh), 2700, 2150, 1680, 1635. ^{1}H-NMR (CD_3OD) δ : 7.48(1H, d, J=1.0 Hz, 3-H), 5.48(1H, t-like, 7-H), 5.26(1H, d, J=6.4 Hz, 1-H), 4.72(1H, d, J=7.8 Hz, 1'-H), 3.89(1H, dd, J=12.0 and 1.7 Hz, 6'-Ha), 3.65(1H, dd, J=12.0 and 5.4 Hz, 6'-Hb), 3.40(1H, dd, J=9.0 and 8.8 Hz, 3'-H), 3.27(1H, dd, J=9.0 and 8.1 Hz, 4'-H), 3.23(1H, dd, J=8.1 and 7.8 Hz, 2'-H), 3.14(1H, br td, J=7.8 and 6.8 Hz, 5-H), 2.73(1H, br dd, J=15.9 and 7.8 Hz, 6-Ha), 2.62(1H, br dd, J=7.6 and 6.4 Hz, 9-H), 2.09(1H, ddq, J=15.9, 6.8 and 2.2 Hz, 6-Hb), 1.82(3H, br s, 10-H_3). ^{13}C-NMR (CD_3OD) δ : 171.00(s, 11-C), 151.27(d, 3-C), 140.26(s, 8-C), 127.88(d, 7-C), 113.04(s, 4-C), 100.05(d, 1'-C), 97.62(d, 1-C), 78.42(d, 5'-C), 78.06 (d, 3'-C), 74.92(d, 2'-C), 71.71(d, 4'-C), 62.88(t, 6'-C), 49.77(d, 9-C), 39.82(t, 6-C), 35.93(d, 5-C), 16.30(q, 10-C). Anal. Calcd. for $C_{16}H_{22}O_9 \cdot 1/4$n-PrOH: C, 53.63; H, 6.19. Found: C, 53.88; H, 6.48.

10-Deoxygeniposide 11-GABA amide (<u>30</u>)

A solution of <u>3a</u> (41.4 mg), HOBT (31.1 mg) and EDCI (26.4 mg) in anhydrous THF (1.0 ml) was stirred for 1 hr at room temperature. To the mixture was added a solution of Me-GABA·HCl (18.0 mg) and DMAP (30.9 mg) in anhydrous acetonitrile (1.0 ml) with ice-cooling. The mixture was stirred for 1 hr at the same temperature and subsquently overnight at room temperature. The reaction mixture was concentrated <u>in vacuo</u> and the residue purified by PLC (MeOH - $CHCl_3$ - HCOOH 30 : 65 : 5). The band around Rf 0.50 gave a syrup of 10-deoxygeniposide 11-GABA amide

methyl ester (48.1 mg).

^{1}H-NMR (CD_3OD, 300 MHz) δ_H: 7.14(1H, d, J=1.2 Hz, 3-H), 5.46(1H, quint, J=1.4 Hz, 7-H), 5.26(1H, d, J=6.1 Hz, 1-H), 4.69(1H, d, J=7.8 Hz, 1'-H), 3.88(1H, dd, J=12.3 and 1.8 Hz, 6'-H), 3.65(1H, s, 1"-OCH_3), 3.47(1H, dd, J=12.4 and 5.2 Hz, 6'-H), 3.38(1H, t, J=8.8 and 7.7 Hz, 3'-H), 3.32 - 3.17(7H, m, 2',4',5',5, 4"-H and NH), 2.74 - 2.62(2H, m, 6- and 9-H), 2.36(2H, t, J=7.4 Hz, 2"-H), 1.99(1H, ddquint, J=15.8, 4.1 and 1.9 Hz, 6-H), 1.82(2H, quint, J=7.1 Hz, 3"-H), 1.81(3H, br s, 10-H). FAB-MS m/z: 458 $[M+H]^+$

The above methyl ester (280 mg) was hydrolyzed in MeOH (5.0 ml) with 0.4N aq. NaOH (5.0 ml) for 2 hr at room temperature. Neutralization, purification by PLC (Rf 0.20, MeOH - $CHCl_3$ 3 : 7) and by column chromatography on activated charcoal (720 mg) (MeOH as eluant) as described above for 15 afforded colorless needles of 30 (160 mg) which were recrystallized from n-PrOH.

mp 181-182 ℃. UV λ_{max} (MeOH) nm (log ε): 230.5(3.97). IR ν_{max} (KBr) cm^{-1}: 3410, 3208(sh), 1710, 1656, 1610, 1512. ^{1}H-NMR (CD_3OD) δ : 7.15(1H, d, J=1.0 Hz, 3-H), 5.46(1H, t-like, N-H), 5.26(1H, d, J=6.1 Hz, 1-H), 4.70(1H, d, J=7.8 Hz, 1'-H), 3.88(1H, dd, J=11.7 and 1.0 Hz, 6'-Ha), 3.65(1H, d, J= 11.7 and 5.1 Hz, 6'-Hb), 3.27(2H, br t, J=7.1 Hz, 1"-H), 3.23(1H, dd, J=8.1 and 7.8 Hz, 2'-H), 2.69(1H, brq, J=7.8 Hz, 6 β -H), 2.67(1H, br t, J=7.3 Hz, 9-H), 2.33(2H, t, J=7.1 Hz, 3"-H), 2.01(1H, br d quintet, J=15.6 and 2.2 Hz, 6 α -H), 1.81(3H, br s, 10-H_3). ^{13}C-NMR (CD_3OD) δ : 177.06(s, 4"-C), 170.49(s, 11-C), 147.73(d, 3-C), 140.33(s, 4-C), 127.37(d, 7-C), 116.54(s, 8-C), 99.88(d, 1'-C), 96.89 (d, 1-C), 78.40(d, 5'-C), 78.01(d, 3'-C), 74.92(d, 2'-C), 71.69(d, 4'-C), 62.86(t, 6'-C), 50.60(d, 9-C), 39.94(t, 1"-C), 39.21(t, 6-C), 35.37(d, 5-C), 32.40(t, 3"-C),25.91(t, 2"-C), 16.19(q, 10-C). Anal. Calcd. for $C_{20}H_{29}O_{10}N$: C, 54.17; H, 6.59; N, 3.16. Found: C, 53.99; H, 6.65; N, 2.96.

10-Deoxygeniposide 11-GABA-BSA conjugate (28)

BSA (223.2 mg) and 30 (60 mg) were condensed using EDCI (28.5 mg) acccording to the same procedure as described for 13. Dialysis followed by lyophilization provided 28 (240.0 mg). The 30 /BSA ratio of the conjugate 28 was determined by the aforementioned colorimetric method to give 11.3 mole of 30 / mole of BSA.

2',3',4'-Triacetyl-6'-trityl-10-deoxygeniposide (33)

A solution of 10-deoxygeniposide (3b) (50 mg) and trityl pyridinium fluoroborate (152.4 mg) in a mixed solvent of anhydrous acetonitrile and THF (each 0.5 ml) was stirred overnight at room temperature. The mixture was in turn acetylated overnight at 4℃ with acetic anhydride (0.5 ml) pyridine (1.0 ml). The resulting mixture was diluted with ice-cold water and extracted with $CHCl_3$. The organic layer was washed successively with 1N HCl, sat. aq. $NaHCO_3$ and water, dried

and concentrated in vacuo. The residue was purified by PLC (Rf 0.46, AcOEt - benzene 2 : 8) and the crystalline product was recrystallized from EtOH to yield colorless needles of 33.
mp 190 - 193 ℃. UV λ_{max} (MeOH) nm (log ε): 212.0(4.44), 219(4.31), 228.5(4.29). IR ν_{max} (KBr) cm^{-1}: 2940, 1758, 1704, 1635. ^{1}H-NMR ($CDCl_3$) δ : 7.46 - 7.20(15H, m, 15 × aromatic H), 7.41(1H, d, J=1.5 Hz, 3-H), 5.52(1H, quintet-like, 7-H), 5.30 - 5.05(3H, m, 2'-, 3'-, and 4'-H), 5.21(1H, d, J=6.4 Hz, 1-H), 4.92(1H, d, J=7.6 Hz, 1'-H), 3.71(3H, s, 11-OC$\underline{H}_3$), 3.59(1H, ddd, J=9.8, 4.6 and 2.0 Hz, 5'-H), 3.28(1H, dd, J=10.5 and 4.6 Hz, 6'-Ha), 3.19(1H, ddd, J=7.8, 6.4 and 1.5 Hz, 5-H), 3.06(1H, dd, J=10.5 and 4.6 Hz, 6'-Hb), 2.88 - 2.70(1H, m, 6-H), 2.69(1H, dd, J=7.8 and 6.4 Hz, 9-H), 2.20 - 2.13(1H, m, 6-H), 2.01 and 1.99(each 3H, s, OCOC$\underline{H}_3$), 1.88(3H, br s, 10-H_3), 1.71(3H, s, OCOC$\underline{H}_3$).
Anal. Calcd. for $C_{42}H_{44}O_{12}$: C, 68.10; H, 5.99. Found: C, 67.87; H, 5.99.

6'-Carboxy-2',3',4'-triacetyl-10-deoxygeniposide (34)

A solution of 33 (900 mg) in 80% aqueous AcOH (35 ml) was stirred at 80 ℃ for 30 min. The reaction mixture was poured on iced water and was extracted with $CHCl_3$. The extracts were washed with sat. aq. $NaHCO_3$ and sat. brine and dried. Evaporation in vacuo of the solvent gave a residue which contained 2',3',4'-triacetyl-10-deoxygeniposide together with 2',3',6'-triacetyl-10-deoxygeniposide as a minor product. The mixture was employed without purification in the subsequent step.
To a solution of the detritylated products in acetone (10 ml) was added Jones reagent (1.36 ml) at -70 ℃ with stirring for 10 min. Stirring was continued at room temperature for 6 hr. After decomposition of the excess reagent with i-PrOH, the reaction mixture was diluted with iced water and extracted with $CHCl_3$. The organic layer were extracted with sat. aq. $NaHCO_3$ and the alkaline layer was acidified with 1N HCl and extracted again with $CHCl_3$. The extracts were washed with brine, dried and concentrated in vacuo. The residue (445.7 mg) was recrystallized from benzene gave a colorless needles of 34.
mp 113 - 115 ℃. UV λ_{max} (MeOH) nm (log ε): 234.3(3.90). IR ν_{max} (KBr) cm^{-1}: 3430, 1758, 1703, 1639. ^{1}H-NMR ($CDCl_3$) δ : 7.39(1H, d, J=1.0 Hz, 3-H), 6.70 - 6.10(1H, br m, 6'-O$\underline{H}$), 5.44(1H, br t-like, 7-H), 5.37 - 5.19(2H, m, 3'- and 4'-H), 5.25(1H, d, J=4.6 Hz, 1-H), 5.10 - 4.98(1H, m, 2'-H), 4.96(1H, d, J=7.8 Hz, 1'-H),4.18 - 4.05(1H, m, 5'-H), 3.71(3H, s, 11-OC$\underline{H}_3$), 3.13(1H, td-like, J=8.1 and 4.4 Hz, 5-H), 2.83 - 2.72(1H, m, 9-H), 2.73(1H, br d, J=16.6 Hz, 6-Ha), 2.12(1H, br d, J=16.6 Hz, 6-Hb), 2.05, 2.03 and 1.97(each 3H, s, 3 × OCOC$\underline{H}_3$), 1.76(3H, br s, 10-H_3). ^{13}C-NMR ($CDCl_3$) δ : 170.15 and 169.84(each s, O$\underline{C}$OCH$_3$), 169.76(s, 6'-C), 169.25(s, O$\underline{C}$OCH$_3$), 167.67(s, 11-C), 150.74(d, 3-C), 137.34(s, 4-C), 127.15(d, 7-C), 112.80(s, 8-C), 96.11(d, 1'-C), 95.31(d, 1-C), 72.22(d, 5'-C), 71.86(d, 3'-C), 70.59(d, 2'-C), 69.20(d, 4'-C), 51.28(q, 11-O$\underline{C}$H$_3$), 49.16(d, 9-C), 38.29(t, 6-C),

32.98(d, 5-C), 20.55(q, OCO$\underline{C}H_3$), 20.51, 20.26 and 15.25(each q, 3 × OCO$\underline{C}H_3$). Anal. Calcd. for $C_{23}H_{28}O_{13}$: C, 53.91; H, 5.51. Found: C, 53.70; H, 5.54.

2',3',4'-Triacetyl-10-deoxygeniposide 6'-GABA amide methyl ester (36)

A solution of 34 (855.6 mg), HOBT (451.2 mg) and EDCI (384.1 mg) in anhydrous THF (10 ml) was stirred for 1 hr at room temperature. To the ice-cold mixture was added a solution of Me-GABA·HCl (279.7 mg) and DMAP (489.5 mg) in anhydrous acetonitrile (5.0 ml) and the resulting mixture was stirred for 1 hr. Stirring was continued overnight at room temperature. Removal of the solvent gave a residue which was purified by PLC (Rf 0.50, acetone - $CHCl_3$ 2 : 8). The desired product 36 (549.6 mg) was obtained as colorless needles after recrystallization from aqueous MeOH.

mp 134 - 136 ℃. $[\alpha]_D$ -28.25° (c, 1.03, MeOH). UV λ_{max} (MeOH) nm (log ε): 238.2(4.16).

IR ν_{max} (KBr) cm^{-1}: 3400, 3360, 2950, 1758, 1710, 1705, 1667, 1645. ^{1}H-NMR (CD_3OD) δ : 7.40(1H, d, J=1.2 Hz, 3-H), 6.52(1H, br t, J=5.7 Hz, CON$\underline{H}$), 5.49(1H, br s, 7-H), 5.31(1H, t, J=9.3 Hz, 4'-H), 5.15(1H, d, J=5.4 Hz, 1-H), 5.11(1H, t, J=8.8 Hz, 3'-H), 5.0(1H, dd, J=9.2 and 8.1 Hz, 2'-H), 4.94(1H, d, J=8.1 Hz, 1'-H), 3.93(1H, J=9.8 Hz, 5'-H), 3.71(3H, s,11-OC$\underline{H}_3$), 3.69(3H, s, 1"-OC$\underline{H}_3$), 3.30(2H, qd, J=6.6 and 1.0 Hz, 4"-H_2), 3.16(1H, tdd, J=8.1, 5.1 and 1.0 Hz, 5-H), 2.87 - 2.67(2H, m, 6- and 9-H), 2.37(2H, t, J=7.3 Hz, 2"-H_2), 2.20 - 2.07(1H, m, 6-Hb), 2.06, 2.02 and 1.98(each 3H, s, 3 × OCOC$\underline{H}_3$), 1.84(2H, quintet, J=6.8 Hz, 3"-H_2), 1.82(3H, br s, 10-H_3). ^{13}C-NMR (CD_3OD) δ : 173.56(s, 1"-$\underline{C}OOCH_3$), 169.81, 169.67, 169.18(each s, 3 × $\underline{C}OCH_3$), 167.36(s, 11-$\underline{C}OOCH_3$), 166.36(s, 6'-$\underline{C}ON$), 150.72(d, 3-C), 137.58(s, 8-C), 127.44(d, 7-C), 112.62(s, 4-C), 96.59(d, 1-C), 96.30(d, 1'-C), 72.88 (d, 5'-C), 71.73(d, 3'-C), 70.66(d, 2'-C), 69.55(d, 4'-C), 51.69(q, 1"-O$\underline{C}H_3$), 51.20(q, 11-O$\underline{C}H_3$), 49.01 (d, 9-C), 38.51(t, 4"-C), 38.46(t, 6-C), 33.64(d, 5-C), 31.18(t, 2"-C), 24.30(t, 3"-C), 20.63, 20.55 and 20.31(each q, 3 × OCO$\underline{C}H_3$), 15.12(q, 10-C). Anal. Calcd. for $C_{28}H_{37}O_{14}N$: C, 54.99; H, 6.10; N, 2.29. Found: C, 54.76; H, 6.12; N, 2.27.

10-Deoxygeniposide 6'-GABA amide (35)

Methyl ester 36 (400 mg) was hydrolyzed in MeOH (5.0 ml) with 0.4N aq. NaOH (5.0 ml) for 3 hr under stirring at room temperature. The mixture was worked up as described above for the preparation of 15. The crude products were chromatographed on activated charcoal (1.2 g). Elution with MeOH afforded 35 (288 mg) as a white amorphous foam.

^{1}H-NMR (CD_3OD) δ : 7.47(1H, d, J=1.0 Hz, 3-H), 5.50(1H, br quintet-like, 7-H), 5.17(1H, d, J=6.6 Hz, 1-H), 4.76(1H, d, J=7.8 Hz, 1'-H), 3.74(1H, d, J=9.3 Hz, 5'-H), 3.55(1H, dd, J=9.5 and 8.8 Hz, 4'-H), 3.43(1H, dd, J=9.0 and 8.6 Hz, 3'-H), 3.35 - 3.24(3H, m, 2'- and 4"-H_2), 3.16(1H, q, J=7.8 Hz,

2.82 - 2.55(2H, m, 6- and 9-H), 2.33(2H, t, J=7.3 Hz, 2"-H_2), 2.01(1H, br d, J=16.1 Hz, 6-Hb), 1.90 - 1.68(5H, m, 3"-H_2 and 10-H_3). FAB-MS m/z: 490 $[M+H]^+$

10-Deoxygeniposide 6'-GABA-BSA conjugate (31)

BSA (178.5 mg) and 35 (51 mg) were condensed using EDCI (22.8 mg) according to the same procedure as described for 13. Dialysis followed by lyophilization provided 31 (190.6 mg). The 35 /BSA ratio of the conjugate 31 was determined by the aforementioned colorimetric method to give 9.6 mole of 35 / mole of BSA.

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