[Chem. Pharm. Bull. 19(7)1438—1443(1971)]

UDC 547.597.02 : 547.918.02

Studies on Monoterpene Glucosides and Related Natural Products. XV.[1] Confirmation of the Absolute Configuration of Catalpol[2]

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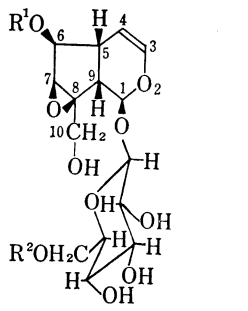
(Received February 9, 1971)

The absolute configuration at C-6 of catalpol (**1**) was established by the chemical conversion of the glucoside to tetrahydroaucubin-B hexaacetate (**16**). Information was also obtained on the configuration at C-8.

Catalpol (**1**) is an iridoid glucoside isolated from the genuses of Catalpa,[4] Plantago, and Buddleja.[5] It also occurs in nature as esters, such as catalposide (**2**).

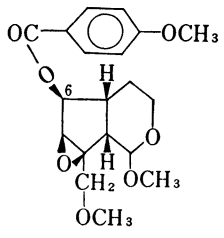
Bobbitt and his co-workers proposed the absolute structure (**1**) of catalpol.[6,7] However, they deduced the configuration at C-6 by application of the Karplus equation to the coupling constants of the C-6 proton signal in the nuclear magnetic resonance (NMR) spectra of compounds (**3**) and (**4**), both of which were derived from catalposide (**2**).[7]



1 : $R^1=R^2=H$

2 : R^1 = HO-(C$_6$H$_4$)-CO-, $R^2=H$

5 : $R^1=H$, R^2 = (C$_6$H$_5$)-CH=CH-CO-



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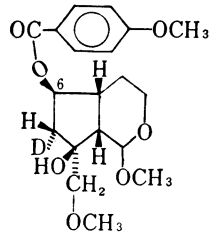

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Chart 1

1) Part XIV: H. Inouye and Y. Nakamura, *Tetrahedron*, **27**, 1959 (1971).
2) A preliminary report of part of this work has been published. I. Kitagawa, H. Hino, T. Nishimura, E. Mukai, I. Yosioka, H. Inouye and T. Yoshida, *Tetrahedron Letters*, **1969**, 3837.
3) Location: *Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto.*
4) V. Plovier, *Compt. Rend.*, **224**, 670 (1947); K. Kimura, T. Okuda and T. Takano, *Yakugaku Zasshi*, **83**, 635 (1963).
5) R. B. Duff, J.S.D. Bacon, C.M. Mundie, U.C. Farmer, J.D. Russel and A.R. Forrester, *Biochem. J.*, **96**, 1 (1965).
6) J.M. Bobbitt, D.W. Spiggle, S. Mohboob, H. Schmid and W. von Philipsborn, *J. Org. Chem.*, **31**, 500 (1966).
7) J.M. Bobbitt, D.E. Kiely, A.Y. Lam and E.I. Snyder, *J. Org. Chem.*, **32**, 1459 (1967).

Recently, Yosioka and his co-workers isolated a new glucoside, picroside I, from the rhizomes and roots of *Picrorhiza kurooa* ROYLE, and concluded that it was 6′-O-*trans*-cinnamoylcatalpol (**5**). Then they noticed that the basis of the assignment of the configuration at C-6 in compound(**3**)by Bobbitt, *et al.* was doubtful.[2,8] Professor Yosioka's suggestion on this point prompted us to study the stereostructure of catalpol (**1**) by correlation with aucubin[9] of known absolute structure. Part of the results have been published in a preliminary form in collaboration with Yosioka's group.[2] This paper reports our part of the work in detail.

Reduction of dihydrocatalpol hexaacetate (**6**)[6] with lithium aluminum hydride in ether–tetrahydrofuran mixture followed by acetylation gave three products: hexaacetate (**7**), $C_{27}H_{38}O_{16}$, a syrupy major product, heptaacetate (**8**), $C_{29}H_{40}O_{17}$, a syrup and diacetate (**9**), $C_{13}H_{18}O_6$–H_2O, mp 118—120°.

The structures of these compounds were inferred as follows: The NMR spectrum of the major product (**7**) shows a signal due to the C-10 methylene group at δ 4.23 as a diffuse singlet and six acetoxyl signals at δ 2.00—2.10, indicating that this substance was formed by the reductive cleavage of the epoxide ring of dihydrocatalpol resulting in the formation of a tertiary hydroxyl group. Accordingly, we can assume that this compound has structure (**7**). On the other hand, the NMR spectrum of compound (**8**) shows seven acetoxyl signals at δ 1.98—2.10, which led us to assume that this compound is produced by the fission of the epoxide ring resulting in the formation of a secondary hydroxyl group. Compound (**9**) shows NMR signals at δ 3.78 (1H, d,d, J_1=10, J_2=2 Hz) and at δ 4.29 (1H, d, J=10 Hz), which seem to be due to the C-10 methylene protons from their coupling constants and splitting patterns. In addition, there is one-proton multiplet at δ 5.10, attributed to the C-6 proton and a one-proton doublet at δ 5.30 (J=5 Hz), attributed to the C-1 proton. The absence of any other signal in the range of δ 4.30—5.10 suggests that this compound (**9**) does not contain the glucose moiety. The singlet at δ 2.03 due to the acetoxyl group overlaps the signals of the methylene and methine protons. Therefore, from these spectral data alone, it was uncertain whether this compound contains two or three acetoxyl groups. Analytical values on this compound were also in good agreement with the formula for triacetate, $C_{15}H_{22}O_8$, so previously we proposed structure (**9a**)[2] for this compound. However, we found that when the NMR spectrum of **9** was measured in d_6-dimethylsulfoxide containing pyridine, the acetoxyl signal was clearly split into two peaks of equal intensity around δ 2.03, and that no signal in the spectrum of this compound disappeared on addition of D_2O. The results indicating the presence of two acetoxyl groups and the absence of any free hydroxyl group in compound (**9**) showed that the compound did not have structure (**9a**).

Compound (**9**) was treated with sodium methoxide in methanol to give monoacetate (10), $C_{11}H_{16}O_5 \cdot 1/2\ H_2O$, mp 99—100°. The NMR spectrum of **10** shows a singlet at δ 2.03 due to an acetoxyl group, a double-doublet at δ 3.77 (J_1=10, J_2=2 Hz), a doublet at δ 4.20 (J=10 Hz) due to C-10 methylene protons and a doublet at δ 5.29 (J=5.5 Hz) due to the C-1 proton. On the other hand, in the NMR spectrum of **10**, a multiplet at δ 5.10 due to the C-6 proton of **9** shifts to a higher field to be buried under groups of signals in the range of δ 3.55—4.40 including those due to C-3 methylene protons. These NMR data together with the observed regeneration of **9** by acetylation of **10** by the conventional method indicated that only the acetoxyl group at C-6 was split off by the methanolysis mentioned above.

On the other hand, treatment of hexaacetate (**7**) with sodium methoxide in a similar manner to that described above, followed by hydrolysis with β-glucosidase (emulsin) gave a

8) Bobbitt's group deduced the configuration at C-6 in (3) on erroneous ground, but their conclusions on the stereostructure were correct, presumably because they also studied the NMR spectrum of the compound (4).

9) N. Masaki, M. Hirabayashi, K. Fuji, K. Osaki and H. Inouye, *Tetrahedron Letters*, **1967**, 2367.

syrupy monoacetate (**11**), $C_{11}H_{16}O_5 \cdot 1/2\ H_2O$. The NMR spectrum of this compound (**11**) shows a singlet at δ 2.05 due to an acetoxyl group, a double doublet at δ 3.71 ($J_1=10, J_2=2$ Hz), a doublet at δ 4.00 ($J=10$ Hz) due to C-10 methylene protons, a multiplet at δ 5.14 due to the C-6 proton and a doublet at δ 5.35 ($J=5$ Hz) due to the C-1 proton. All these signals of compound (**11**) correspond well to those of 9. But the sharp, one-proton singlet at δ 5.36 in the NMR spectrum of **11** in d_6-dimethylsulfoxide disappears on addition of D_2O. These facts indicate that monoacetate (**11**) has a tertiary hydroxyl group as its sole free hydroxyl group. These and additional considerations with respect to the fact that an acetoxyl group is located on C-6 indicated that the oxygen on C-1 and C-10 in **11** should be present as an ether and that the methyleneoxy group at C-8 should thus have an α-orientation so as to be a part of the ethereal structure. Consequently, the monoacetate should be represented by absolute structure (**11**).

TABLE I. NMR Signals of Compounds (**9**), (**10**) and (**11**) (δ)

	C_{10}–H_2	C_1–H	C_6–H	Ac–H_3
Diacetate (**9**)	3.78 (d.d, $J_1=10$, $J_2=2$) 4.29 (d, $J=10$)	5.30 (d, $J=5$)	5.10 (m)	2.03 (6H)
Monoacetate (**10**)	3.77 (d.d, $J_1=10$, $J_2=2$) 4.20 (d, $J=10$)	5.29 (d, $J=5.5$)	4.40—3.55 (m, 3H)	2.03 (3H)
Monoacetate (**11**)	3.71 (d.d, $J_1=10$, $J_2=2$) 4.00 (d, $J=10$)	5.35 (d, $J=5$)	5.14 (m)	2.05 (3H)

d.d,: double doublet, d: doublet, m: multiplet

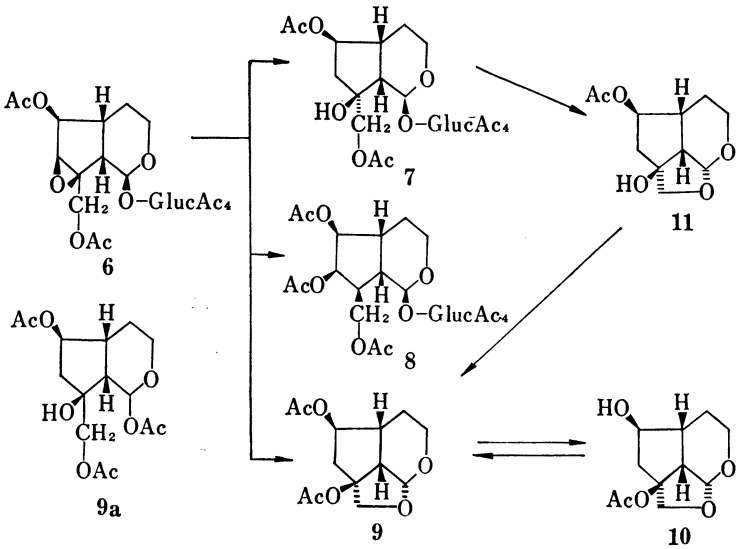

Chart 2

On the other hand, monoacetate (**11**) was acetylated by the conventional method to give colorless plates of diacetate, mp 119—120°, which was identified with **9**. Accordingly, compounds (**9**) and (**10**) should be represented by the absolute structure shown in Chart 2.

The structure of an ether of the type (C-1)-O-(C-10) of compounds (**9**), (**10**) and (**11**) was also supported by the fact that the NMR signals due to C-10 methylene and C-1 acetal protons of these compounds show almost constant chemical shifts independent of acetylation [(**10**)→(**9**),

(**11**)→(**9**)] and deacetylation [(**9**)→(**10**)]. Moreover, the long range coupling (J=2 Hz) of one part of the AB type signal due to C-10 methylene protons of these compounds (**9**), (**10**) and (**11**) is compatible with the assumption that this methylene group is in a rigid system. (*cf.* Table I).

Bobbitt and his colleagues[6] have already proved by conversion of **2** to **12** that the methyleneoxy group at C-8 of catalposide (**2**) has an α-orientation. Inouye and Arai[10] deduced the configuration at C-8 of the aglucone of tetrahydromonotropein (**13**) from the fact that the furan derivative (**14**) was formed on treatment of this compound with acid. The experimental results obtained here provide confirmation for these assumptions on the absolute configuration at C-8 of both **12** and **13**.

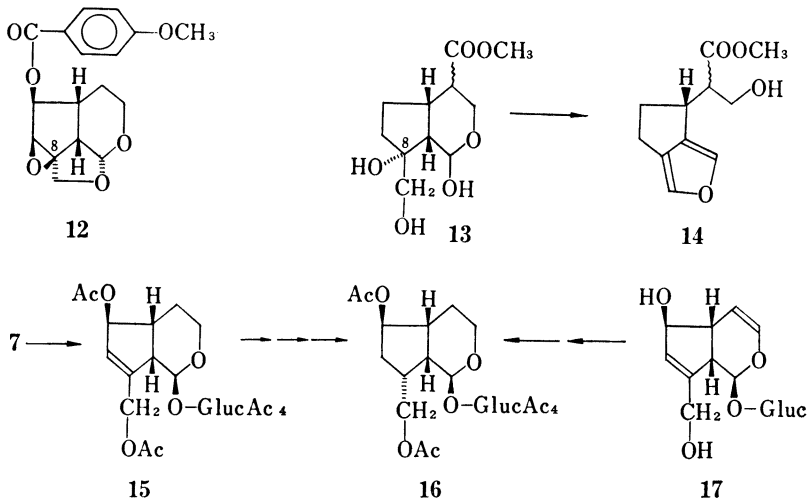

Chart 3

Hexaacetate (**7**) was treated with phosphorous oxychloride and pyridine giving the dehydrated compound (**15**), $C_{27}H_{36}O_{15}$. In the NMR of **15**, besides signals at δ 2.00—2.08 due to six acetoxyl groups there are signals assignable to C-10 methylene protons, shifted downfield from δ 4.23 (in **7**) to δ 4.71 and a diffuse singlet at δ 5.83 due to a newly formed vinyl proton. From these observations we concluded that the dehydrated product has structure (**15**). Next we obtained a dihydro derivative of **15** by catalytic hydrogenation. It was anticipated that on reduction of **15**, hydrogenolysis might readily occur owing to the presence of two allylic acetoxyl groups.[11] Therefore, **15** was first deacetylated with sodium methoxide in methanol and then subjected to catalytic hydrogenation over Adams catalyst in ethanol followed by reacetylation to furnish the expected hydrogenation product (**16**) in good yield, $C_{27}H_{38}O_{15}$, mp 112—113°, $[\alpha]_D$ −73.1° ($CHCl_3$). This compound (**16**) was identified with tetrahydroaucubin-B hexaacetate (**16**) of known structure.[9]

Thus the hydroxyl group at C-6 of catalpol (**1**) was conclusively proved to be β-oriented as in aucubin (**17**).

10) H. Inouye and T. Arai, *Chem. Pharm. Bull.* (Tokyo), **16**, 1019 (1968).

11) On catalytic hydrogenation of aucubin hexaacetate, the formation of a considerable amount of hydrogenolysis product besides the hydrogenation product has been reported. *cf.* Y. Iwanami, Y. Hotta, T. Kubota, S. Fujise, T. Ishikawa and H. Uda, *Nippon Kagaku Zasshi*, **76**, 77 (1955).

Experimental[12]

Reduction of Dihydrocatalpol Hexaacetate (6) with Lithium Aluminum Hydride——A solution of dihydrocatalpol hexaacetate (**6**) (4.2 g) in abs. tetrahydrofuran (80 ml) was added dropwise to a suspension of $LiAlH_4$ (4.5 g) in abs. ether (60 ml) and the reaction mixture was refluxed for 1 hr. After decomposition of the excess reagent with AcOEt, saturated aqueous Na_2SO_4 was added to the reaction mixture resulting in formation of a white precipitate. The supernatant was decanted off and the residue was triturated repeatedly with hot EtOH. The EtOH solution was adjusted to pH 4 with an ion exchange resin (Amberlite IR 120, H-form) and evaporated *in vacuo* to give a brown syrup. This was acetylated with 15 ml each of Ac_2O and pyridine in the usual way. The resulting pale yellow syrup (2.1 g) was chromatographed on silica gel (20 g) using ether as eluent and 3 ml fractions were collected.

Diacetate (9)——The residues from fractions No. 10—18 were combined and recrystallized from EtOH and then from ether–petr. ether to give colorless plates of diacetate (**9**), mp 118—120° (83 mg). IR ν_{max}^{Nujol} cm^{-1}: 1725, 1040, 970, 960, 940. *Anal.* Calcd. for $C_{13}H_{18}O_6 \cdot H_2O$: C, 54.16; H, 6.99. Found: C, 54.44; H, 6.71.

Heptaacetate (8)——Fractions No. 22—30 were combined and evaporated to give a colorless syrup of **8** (263 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1230—1210, 1035. *Anal.* Calcd. for $C_{29}H_{40}O_{17}$:C, 52.72; H, 6.10. Found: C, 52.59; H, 6.32.

Hexaacetate (7)——Fractions No. 35—47 yielded 901 mg of hexaacetate (**7**) as a colorless syrup. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (weak), 1740 1240—1210. *Anal.* Calcd. for $C_{27}H_{38}O_{16}$: C, 52.42; H, 6.19. Found: C, 52.32; H, 6.37. TLC (ether): diacetate (**9**) *Rf* 0.72, heptaacetate (**8**) *Rf* 0.36, hexaacetate (**7**) *Rf* 0.18.

Hydrolysis of Diacetate (9)——To a solution of diacetate (**9**) (210 mg) in abs. MeOH (3 ml) was added 0.1 N NaOMe (1 ml). The mixture was heated under reflux for 1 hr. After cooling, the solution was neutralized with Amberlite IR 120, and concentrated *in vacuo* to give a brown syrup. This was dissolved in a small volume of solvent mixture (CH_2Cl_2: MeOH 6:4 v/v). The solution was put on a silica gel column (10 g) and eluted with CH_2Cl_2 and 5 ml fractions of eluate were collected. Fractions No. 3—5 were evaporated to give a colorless syrup which was crystallized from ether–petr. ether. Two recrystallizations from the same solvent mixture gave 19 mg of **10** as colorless needles, mp 99—100°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1730, 1240—1210, 1050. *Anal.* Calcd. for $C_{11}H_{16}O_5 \cdot 1/2\ H_2O$: C, 55.68; H, 7.22. Found: C, 56.02; H, 7.36.

Acetylation of 8-Monoacetate (10)——8-Monoacetate (**10**) (19 mg) was acetylated in the usual way and the reaction product was recrystallized from ether–petr. ether to give 16 mg of colorless plates, mp 121—122,° identical (mixed mp, IR (Nujol) and NMR spectra) with **9** derived from **6**.

Hydrolysis of Hexaacetate (7)——To a solution of hexaacetate (**7**) (765 mg) in abs. MeOH (3 ml) was added 0,1 N NaOMe (1 ml) and the mixture was heated under reflux for 1 hr. After cooling, the solution was neutralized with Amberlite IR 120, and the solvent was removed *in vacuo* to give an yellow syrup which was dissolved in 0.1 M acetate buffer (pH 4.5, 15 ml) and subjected to hydrolysis with emulsin (130 mg) prepared from apricot kernels. After standing overnight at 37°, the reaction mixture was poured into MeOH and evaporated *in vacuo* to give a brown residue which was triturated repeatedly with the same solvent. The combined MeOH solutions were concentrated *in vacuo* to afford a brown syrup. This was chromatographed on silica gel (15 g) with AcOEt as eluent and 10 ml fractions were collected. The residue obtained from fractions No. 2—9 was rechromatographed on silica gel (5 g) with CH_2Cl_2 as eluent and 5 ml fractions were collected. Fractions No. 26—30 yielded 52 mg of monoacetate (**11**) as a colorless syrup, which gave a single spot on TLC (ether, *Rf* 0.22). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1728, 1250—1210, 1020. *Anal.* Calcd. for $C_{11}H_{16}O_5 \cdot 1/2\ H_2O$: C, 55.68; H, 7.22. Found: H, 55.52; H, 7.34.

Acetylation of 6-Monoacetate (II)——6-Monoacetate (**II**) (11 mg) was acetylated in the usual manner to give 5 mg of colorless plates, mp 118—120°, identical (mixed mp, IR (Nujol) and NMR spectra) with an authentic sample of diacetate (**9**).

Dehydration of Hexaacetate (7)——To a solution of hexaacetate (**7**) (605 mg) in pyridine (6 ml) was added $POCl_3$ (1 ml). The solution was stood overnight in an ice box. It was then poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed successively with 5% HCl and water and dried over anhyd. $MgSO_4$. Removal of the solvent gave a pale yellow syrup (574 mg) which was chromatagraphed on silica gel (15 g) with ether as eluent. The fractions containing a substanee giving the main spot, *Rf* 0.40, on TLC (ether) were combined and the solvent was removed to give 285 mg of **15** as a colorless syrup. *Anal.* Calcd. for $C_{27}H_{36}O_{15}$: C, 54.00; H, 6.04. Found: C, 53.71; H, 6.28.

12) All mps are given as uncorrected values. Unless otherwise stated NMR spectra were recorded with materials in $CDCl_3$ solution using a Varian A-60 Spectrometer. Chemical shifts are given as δ values with TMS as the internal standard and coupling constants (J) in Hz. IR spectra were measured in a Hitachi EPI-S2 type Infrared Spectrophotometer. Specific rotations were determined with a Rex Photoelectric Polarimeter, Silica gel G acc. to Stahl (Merck) was used for thin layer chromatography (TLC). Materials were located by exposure of the layer to iodine vapour. Silica gel (Mallinckrodt) was used for column chromatography. The petroleum ether used in this experiment boiled in the range of 38—45°.

Catalytic Hydrogenation of 15——To a solution of **15** (265 mg) in abs. MeOH (6 ml) was added 0.1 N NaOMe (0.5 ml) and the mixture was refluxed for 5 min. After cooling, the solution was adjusted to pH 5 with Amberlite IR 120 and the solvent was removed *in vacuo* to give a yellow syrup (165 mg). This was subjected to the catalytic hydrogenation over prereduced PtO_2 (30 mg) in EtOH (8 ml) without further purification. After an uptake of 9.5 ml (1 mole equivalent) of H_2 within 1 hr, the catalyst was filtered off and the filtrate was evaporated *in vacuo* to give a colorless syrup which was acetylated in the usual way. The resulting colorless syrup (226 mg) was crystallized from EtOH–water. Two recrystallization from the same solvent afforded 140 mg of fine colorless needles, mp 112—113°, $[\alpha]_D^{20}$ −73.1° (c=0.8, $CHCl_3$). *Anal* Calcd. for $C_{27}H_{38}O_{15}$: C, 53.82; H, 6.35. Found: C, 54.02; H, 6.47. This substance was identified with tetrahydroaucubin-B hexaacetate (**16**) by the mixed melting point and by comparisons of its IR (Nujol) and NMR spectra with those of the authentic material.

Acknowledgement The authors are grateful to professor I. Yosioka of Osaka University for his suggestions. Thanks are also due to Dr. T. Shingu and Miss M. Ohkawa of this Faculty for NMR determinations, and to the members of the Microanalytical Center of this University for microanalyses.