Tetrahedron Letters No.43, pp. 3837-3840, 1969. Pergamon Press. Printed in Great Britain.

PICROSIDE I : A BITTER PRINCIPLE OF PICRORHIZA KURROOA

Isao Kitagawa, Katsuhiko Hino, Tadashi Nishimura, Etsuko Mukai and Itiro Yosioka

Faculty of Pharmaceutical Sciences, Osaka University

Toyonaka, Osaka, Japan

Hiroyuki Inouye and Takashi Yoshida

Faculty of Pharmaceutical Sciences, Kyoto University

Sakyo-ku, Kyoto, Japan

(Received in Japan 23 July 1969; received in UK for publication 18 August 1969)

From the rhizome and roots of Picrorhiza kurrooa Royle(huhuanglian:胡黄連)(Scrophulariaceae), which grows in Himalayan area and is used as a folk-medicine in India and China, Rastogi et al. isolated[1] a bitter principle kutkin(=6-cinnamoyl-β-D-glucosidyl vanillate) in addition to mannitol and vanillic acid, while Yeh reported[2] the isolation of picrorhizin(=glucosido-vanilloyl glucose), tripalmitin and phytosterol. As described in the present paper, however, we have isolated a new bitter principle, named picroside I(I), along with mannitol and vanillic acid.

Picroside I(I)(amorphous, slightly hygroscopic), $C_{24}H_{28}O_{11}$[3], $[\alpha]_D$-82°(MeOH); IR(nujol, cm^{-1}): 3400～3200(OH), 1660(sh., enol ether), 1705, 1636(conjugated ester), 1605(sh.), 1580, 1495(aromatic); NMR(100Mc, D_2O, τ): 2.36, 3.57(1H each, d., J=16cps.), 2.55(5H, aromatic), gave on $Ba(OH)_2$ hydrolysis, t-cinnamic acid and a crystalline product, $C_{15}H_{22}O_{10}$ mp.205-6°, $[\alpha]_D$-110°(90% EtOH). The NMR decoupling experiment of the hexaacetate(III) derived from the latter led us to assume the product being catalpol(II)[4] and the assumption was verified by direct comparison with the authentic sample[4*] (mp., IR, $[\alpha]_D$, TLC), thus revealing that picroside I is a t-cinnamoyl ester of catalpol(II).

Dihydrocatalpol(V) gave a ditrityl derivative(VII); NMR: centered at 2.68(30 H, m.), while tetrahydropicroside I(IV), $C_{24}H_{32}O_{11}$; IR: 1725; NMR: 7.9～8.3(4H, m.), 6.98(4H, A_2B_2, $-C\underline{H}_2-C\underline{H}_2-C_6H_5$), prepared by catalytic hydrogenation(Pd-C) of I, afforded only a monotrityl deriv.(VI); NMR: 2.76(br. s., $W_{\frac{1}{2}}$=12 cps., 20 H), under the same reaction condition(TrCl-Py.). The NMR examination(Table 1) of I, II, IV, V, and catalposide(VIII)[4] discloses that each one of primary alcoholic functions of I and IV are esterified as shown by the signals due to the methylene protons appearing in the lower region(5.2～5.7 τ) compared to the corresponding signals in II, VIII, and V.

Table 1 (τ values at 100 Mc in D_2O)

compounds	5.2 ~ 5.7 region	5.7 ~ 6.7 region
I	3H(C_{10}-H, $C_{6'}$-H_2)	7H(C_6 C_7 C_{10}-H ; C_2·C_3·C_4·C_5·-H)
II	1H(C_{10}-H)	9H(C_6 C_7 C_{10}-H ; C_2·C_3·C_4·C_5·-H ; $C_{6'}$-H_2)
VIII	1H(C_{10}-H)	8H(C_7 C_{10}-H ; C_2·C_3·C_4·C_5·-H ; $C_{6'}$-H_2)
IV	4H(C_6 C_{10}-H ; $C_{6'}$-H_2)	8H(C_3-H_2 ; C_7 C_{10}-H ; C_2·C_3·C_4·C_5·-H)
V	2H(C_6 C_{10}-H)	10H(C_3-H_2 ; C_7 C_{10}-H ; C_2·C_3·C_4·C_5·-H ; $C_{6'}$-H_2)

A pentaanisoyl deriv.(IX), $C_{64}H_{62}O_{21}$; IR(CCl_4): 1735, 1725(sh.) of IV was converted to a desglucosyl deriv.(X), $C_{26}H_{28}O_9$(M^+: 484); IR(CCl_4); 1718, 1608, 1510, 1268, 1252; NMR: 3.10, 2.00(4H each, d., J=9 cps.), 6.16(6H, s), 6.64(3H, s) by treatment with p-TsOH in MeOH-benzene. The formulation(X) has been rationalized by the NMR decoupling experiment, so that the location of dihydrocinnamoyl group in IV has been demonstrated at one of the hydroxyls in the glucosyl moiety.

In addition, the consumption (2 mole) in the periodate titration of IV as well as V secured the location at 6', thus establishing the structure of picroside I as 6'-O-t-cinnamoyl-catalpol(I).

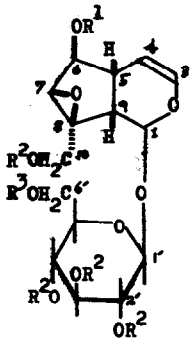
To ascertain C_6-βOH in catalpol(II), Bobbitt et al.[4b] applied the Karplus equation[5] in the NMR assignment especially of C_6- and C_7-H. As shown by Tori et al.[6] and Geissman et al.[7], the coupling constant between an epoxidic and an adjacent protons in the five membered ring would not follow the Karplus equation. It, therefore, has become of interest to re-examine the NMR data of the catalpol derivatives.

The decoupling experiment of X(Table 2) comfirmed that $J_{5,6}$ $J_{6,7}$ are 9.8 and 1.2 cps. respectively contrary to the Bobbitt's presentation[4b]. Similar assignments have also been found true in cases of III, XI $C_{34}H_{38}O_{16}$, XII $C_{34}H_{42}O_{16}$ and XIII.

Table 2 (Decoupling experiment of X, τ values at 100 Mc in $CDCl_3$)

irradiated at / decoupled	C_5-H	C_6-H	C_7-H	C_9-H(7.35, q., J=7 & 9)
C_5-H 7.68(m.)		simplified		
C_6-H 4.50(q., J=9.8 & 1.2)	s.-like $W_{\frac{1}{2}}$=2.5		d., J=9	
C_7-H 6.24(s.-like, $W_{\frac{1}{2}}$=3.5)		s., $W_{\frac{1}{2}}$=2.5		
C_1-H 5.14(d., J=5)				singlet

Furthermore, an alternate chemical proof of C_6-βOH in II has been accomplished by the following derivation starting from XIII. $LiAlH_4$ reduction of XIII followed by acetylation furnished a triacetate(XIV), $C_{15}H_{22}O_8$[8], a heptaacetate(XV), $C_{29}H_{40}O_7$, and a hexaacetate(XVI)(major product)



I $R^1=R^2=$ H, $R^3=$ t-cinnamoyl **picroside I**

II $R^1=R^2=R^3=$ H **catalpol**

III $R^1=R^2=R^3=$ Ac

VIII $R^1=$ p-OH-benzoyl, $R^2=R^3=$ H **catalposide**

XI $R^1=R^2=$ Ac, $R^3=$ t-cinnamoyl

3,4-dihydro-

IV $R^1=R^2=$ H, $R^3=COCH_2CH_2C_6H_5$

V $R^1=R^2=R^3=$ H

VI $R^1=R^2$(at C_2,C_3,C_4,)= H, R^2(at C_{10})= Tr, $R^3=COCH_2CH_2C_6H_5$

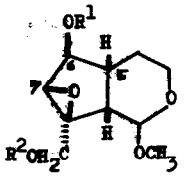
VII $R^1=R^2$(at C_2,C_3,C_4,)= H, R^2(at C_{10}) $=R^3=$ Tr

IX $R^1=R^2=$ anisoyl, $R^3=COCH_2CH_2C_6H_5$

XII $R^1=R^2=$Ac, $R^3=COCH_2CH_2C_6H_5$

XIII $R^1=R^2=R^3=$ Ac

XIX $R^1=R^2$(at C_2,C_3,C_4,)= anisoyl, R^2(at C_{10})$=R^3=$ Tr

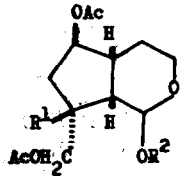


X $R^1=R^2=$ anisoyl

XX $R^1=$ anisoyl, $R^2=$ H

XXI $R^1=$ anisoyl, $R^2=CH_3$

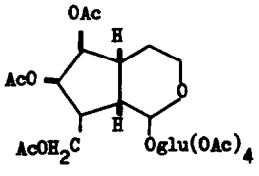
XXII $R^1=$ H, $R^2=CH_3$



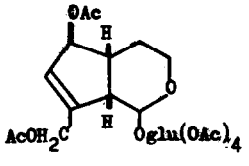
XIV $R^1=$ OH, $R^2=$ Ac

XVI $R^1=$ OH, $R^2=$ glu(OAc)$_4$

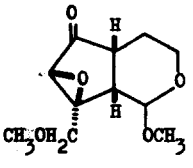
XVIII $R^1=$ H, $R^2=$ glu(OAc)$_4$



XV



XVII



XXIII

$C_{27}H_{38}O_{16}$; NMR: 5.77(diffused s., $-C_{(10)}\underline{H}_2$). Dehydration of XVI with $POCl_3$-Py. yielded XVII, which was further submitted to deacetylation[9] followed by hydrogenation(Pd-C) and re-acetylation to give a product $C_{27}H_{38}O_{15}$ mp.112-3°. The final product was confirmed identical with tetrahydroaucubin hexaacetate(XVIII) whose configuration had already been established[10].

It follows that although the aforementioned NMR explanation on C_5 C_6 C_7-protons in the catalpol derivatives by Bobbitt et al[4]. was inapplicable, the β orientation at C_6 in II is correct.

We have also obtained an additional evidence concerning to the β-epoxide configuration in catalpol. Thus, a tetraanisoyl deriv.(XIX), $C_{85}H_{82}O_{18}$ derived from VII was treated with p-TsOH in MeOH-benzene to give XX, (M^+: 350); IR(CCl_4) :3540, 1708, 1606, 1510, 1275, 1257. Methylation of XX with MeI-Ag_2O in DMF yielded XXI, $C_{19}H_{24}O_7$; IR(CCl_4) : 1715. The coupling constants ($J_{6,7}$ and $J_{5,6}$) in XX and XXI were found similar as in X, and β-OCH_3 orientations at C_1 in XX and XXI were assumed by their $J_{1,9}$ values(d., J=8.5 and 8.0 cps. respectively). Upon CrO_3-Py. oxidation, the deanisoyl deriv.(XXII), mp. 110-2°(M^+: 230), obtainable by MeONa-MeOH treatment of XXI, gave a fairly unstable epoxyketone(XXIII)(M^+: 228); IR(CCl_4); 1755. The epoxy-ketone exhibited a positive Cotton effect in its CD curve at $n\rightarrow\pi^*$ transition($[\theta]_{308^+}$ 16240(max.))[11], which makes sure of the absolute configuration as depicted by XXIII.

The authors wish to express their sincere thanks to Res. Lab. of Takeda Chem. Industries and Mrs. M.Okawa of Kyoto Univ. for NMR measurement, to Dainippon Pharm. Co. and Dr. K.Hozumi of Kyoto Univ. for elemental analyses, and to Dr. K.Kuriyama, Shionogi Pharm. Co. for CD. data and discussion.

REFERENCES

1) R.P.Rastogi, M.L.Dhar: J.Sci. & Ind. Research(India)18B, 219(1959)[C.A. 53, 20294(1959)] and the literatures cited therein.
2) P.Y.Yeh: J.Taiwan Pharm.Assoc., 4 25(1952). [C.A. 48, 14121(1954)]
3) All compounds given with chemical formulae gave satisfactory analytical values.
4) a) J.M.Bobbitt, D.W.Spiggles, S.Mahboob, H.Schmid, W.von Philipsborn: J.Org.Chem., 31 500(1966).
b) J.M.Bobbitt, D.E.Kiely, A.Y.-W.Lam, E.I.Snyder: J.Org.Chem., 32 1459(1967).
*) The authors are greatly indebted to Dr.T.Okuda of Kyoto Univ. for authentic catalpol.
5) M.Karplus: J.Am.Chem.Soc., 85 2870(1963).
6) K.Tori, T.Komeno, T.Nakagawa: J.Org.Chem., 29 1136(1964).
7) T.A.Geissman, W.F.Knaack, Jr., J.O.Knight: Tetrahedron Letters, 1966 1245.
8) The structure was assured by the NMR decoupling experiments.
9) Performed to avoid the hydrogenolysis which occurs in the hydrogenation of aucubin hexaacetate. cf. Y.Iwanami, Y.Hotta, T.Kubota, S.Fujise, T.Ishikawa, H.Uda: Nippon Kagaku Zasshi, 76 77(1955).
10) N.Masaki, M.Hirabayashi, K.Fuji, K.Osaki, H.Inouye: Tetrahedron Letters, 1967 2367, and the literatures cited therein.
11) H.Wehrli, C.Lehmann, P.Keller, J.J.Bonnet, K.Schaffner, O.Jeger: Helv.Chim.Acta, 49 2188(1966).