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Isolation and Structures of Cropapine and Cronupapine, New Glucosides from *Cronura Papirio*

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Two new glucosides, cropapine and cronupapine, have been isolated from the plant *Cronura papirio* and their structures have also been elucidated on the basis of their spectral data coupled with some chemical evidence.

In the course of our searching for bioactive and water soluble substances of the toxic plants *Orchidaceae* including dendrobine, nobiline and related alkaloids,[1-4] we could isolate two new glucosides, cropapine and cronupapine, from the plant *Cronura papirio*. In this communication we wish to describe the isolation and structural determination of these glucosides.

Fresh leaves, flowers and roots of the plant *Cronura papirio* collected at Hiyoshi early in May were immersed in MeOH at room temperature for two months, and then the MeOH extract was concentrated under reduced pressure to leave a greenish brown oil, which was partitioned between water and EtOAc. The water soluble fraction was roughly separated by column chromatography on Develosil ODS using a mixed solvent of MeOH-H_2O (1:1). The fraction containing two new glycosides was further separated by preparative TLC [Kieselgel PF_{254}; $CHCl_3$ - MeOH (7:1)] to afford two new compounds, named cropapine (**1**) and cronupapine (**2**), in 0.0036 and 0.018% yields (based on the weight of the plant 220 g), respectively. Cropapine (**1**)[5] was obtained as a colorless oil, whose FAB mass spectrum exhibited the molecular ion peak at m/z 360 in accord with the molecular formula $C_{17}H_{28}O_8$, and a fragment one at m/z 197 (aglycone peak) formed by loss of a sugar moiety . The IR spectrum of **1** suggests the presence of a hydroxyl group (3370 cm^{-1}) and a CO group (1705 cm^{-1}). The ^1H NMR (CD_3OD) spectrum shows the signals of four olefinic protons [δ 5.94 (H-7), 5.28 ($H\text{-}8_{cis}$), 5.21($H\text{-}8_{trans}$)], two methyl groups [δ 1.40 (H-10), 1.81 (H-9)], one methoxyl group (δ 3.71), and sugar protons (δ 4.36-3.16) which are overlapped with the solvent signals.

On acetylation with Ac_2O-pyridine (room temp, 24 h), cropapine (**1**) was readily converted into the corresponding tetraacetate (**1a**) as a colorless oil [$C_{25}H_{36}O_{12}$ (m/z 528.2203 (M^+)); IR (film) 1760, and 1230 cm^{-1} ; δ ($CDCl_3$) 2.07, 2.04, 2.02, and 2.00]. The ^1H NMR ($CDCl_3$) spectrum of **1a** with the aid of decoupling experiments indicates the presence of the following fragments; C_3-C_5, C_7-C_8, and the glucose whose anomeric proton must be in an axial configuration as judged from the coupling constant (J = 7.8 Hz) between H-1'(δ 4.80) and H-2' (δ 5.02). Furthermore, the E-geometry of the conjugated double bond was based on NOE difference experiments. Particularly, irradiation of the methyl singlet (δ 1.80) of **1a** resulted in 2.2% NOE of the methylene signal (δ 2.17). Therefore, the structure of cropapine is represented by **1**, as seen in Fig. 1.

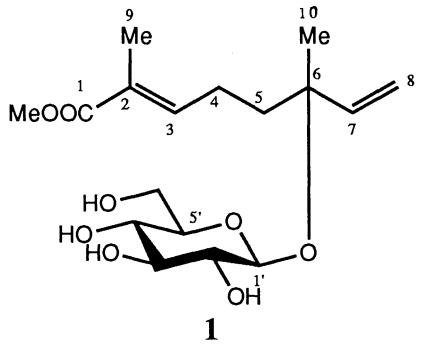

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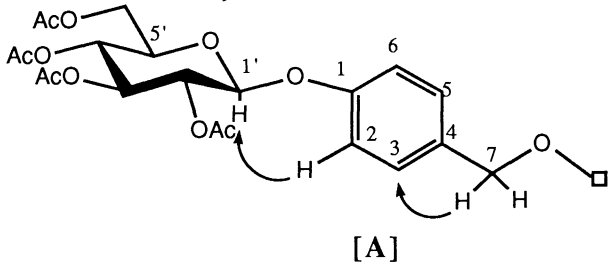
Fig. 1.

Cronupapine (**2**)[6] was isolated as a colorless oil. The high resolution FAB mass spectrum [m/z 515.1530 $(M+Na)^+$] exhibited the molecular formula $C_{24}H_{28}O_{11}$, and the fragment ion peak at m/z 311 was formed by loss of a sugar moiety. The ^1H and ^{13}C NMR (CD_3OD) spectra of **2** suggested the presence of one glucose (δ_H 4.9, ; δ_C 103.01, anomeric carbon), each one of para-substituted and mono-substituted aromatic rings [δ_H 7.25 (2H, d, 8.5 Hz), 7.02 (2H, d, 8.5 Hz), 7.19 (3H, br.m), 7.10 (2H, m)], three isolated methylenes [δ_H 5.04 (s), δ_C 68.74; δ_H 2.96 (d, 17.1 Hz), 2.59 (d, 17.1 Hz), δ_C 44.87; δ_H 2.90 (d, 13.7 Hz), 2.98 (d, 13.7 Hz), 2.92 (d, 13.7 Hz), δ_C 47.07], and two CO groups (δ_C 176.33 and 174.90).

When treated with Ac_2O - pyridine (room temp, overnight), **2** was almost quantitatively converted into the pentaacetate (**2a**) as a colorless oil [$C_{25}H_{36}O_{12}$ (m/z 528.2203 (M^+)) ; IR (film) 3600 - 2800 (br.), 1760, and 1230 cm^{-1}, δ_H ($CDCl_3$) 1.99, 1.97, 1.96, 1.95, and 1.94]. The ^1H NMR spectrum of the pentaacetate is quite similar to that of **2** except for the following points; the NMR signals assignable to the sugar moiety at δ (CD_3OD) 3.50-3.29, and 3.98 in **2** were shifted to δ ($CDCl_3$) 5.00 ($C_{1'}$-H), 5.17 ($C_{2'}$-H), 5.21 ($C_{3'}$-H), 5.07 ($C_{4'}$-H), 3.76 ($C_{5'}$-H), 4.19 ($C_{6'}$-H), and 4.07 ($C_{6'}$-H), respectively, in **2a**. Therefore, one of the five acetoxyl groups must be located at the quaternary carbon (δ_C 80.59). Furthermore, the presence of a carboxyl group was confirmed by methyl ester formation [δ (CD_3OD) 3.56, COOMe] using $TMSCHN_2$ in benzene-MeOH (5 : 1) (room temp, 25 min).

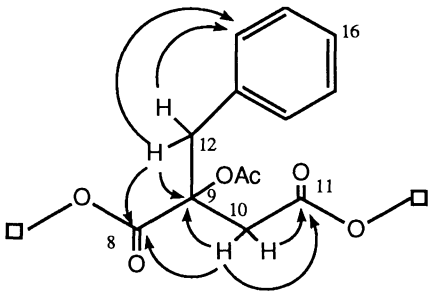
The NOE difference experiments indicated the connectivity between sugar moiety and para-substituted aromatic ring. Irradiation of the C_2-H (δ 6.84) of **2a** resulted in 3.1% NOE of the $C_{1'}$-H (δ 5.00) in the partial structure [A] (see Fig. 2.). In addition, the partial structure [B] was also determined by the COLOC experiments (Fig. 3.) indicating the connectivities of a methylene proton (δ 3.12, C_{10}-H) to two CO carbons (carboxyl group and ester group) [δ 169.4 (C_8) and 173.7 (C_{11})] and a sp^3 carbon [δ 80.59 (C_9)] bearing an oxygen atom, and of another methylene proton (δ 3.34, C_{12}-H) to a CO carbon [δ 169.4 (C_8)], an aromatic carbon[δ 130.48, (C_{14})] and a sp^3 carbon (C_9). Other COLOC interactions are shown in Fig. 2 and 3.



[A]

Irradiation at δ 6.84 (C_2-H; ^{13}C NMR: δ 116.83) of **2a** resulted in 3.1 % NOE of the $C_{1'}$-H (^1H NMR: δ 5.00; ^{13}C NMR: δ 98.82)

Fig. 2. The partial structure [A] of **2a**.



[B]

Fig. 3. The partial structure [B] of **2a**.

Furthermore, the positions of the COOH group (δ 174.90, C_{11}) and the ester group (δ 176.33, C_8) were based on the following chemical evidence; on reduction with $NaBH_4$ and BF_3•Et_2O in THF (room temp, 24 h) followed by acetylation with Ac_2O-pyridine (room temp, overnight),[7] **2a** was converted into the corresponding acetate [IR (film) 1750, and 1230 cm^{-1} ; δ ($CDCl_3$) 2.35 (1H, dt, J=7.32, 14.16 Hz), C_{10}-H; 2.06 overlapped with AcO signals, C_{10}-H; 4.19 (2H, dd, J = 5.37, 7.32 Hz, C_{11}-H_2)], whose NMR spectrum indicates the presence of the partial structure -CH_2-CH_2-OAc, wherein the acetoxymethyl group is originated from the COOH group in **2**. Therefore, the structure of cronupapine is represented by **2** (Fig. 4). Biogenetically, the main skeleton of **2** seems to be formed from each one molecule of phenylpyruvic acid and acetate unit .[8]

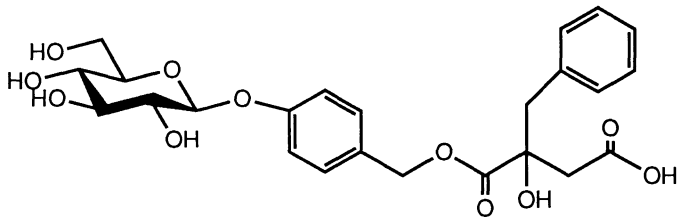


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Fig. 4.

Further studies on biological activities as well as absolute configurations of both cropapine and cronupapine are in progress. These results will be reported in due course.

References

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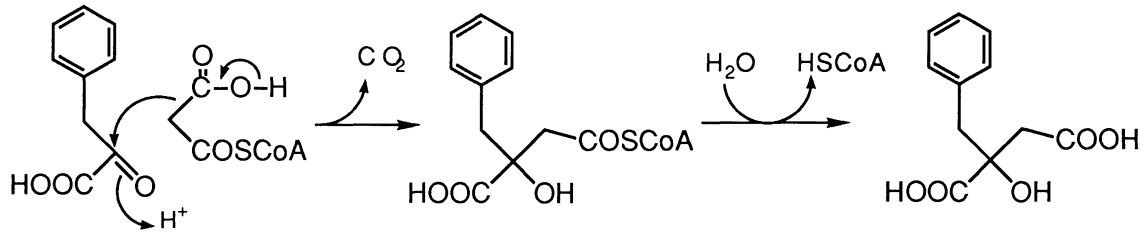
5) Cropapine (**1**) as a colorless oil : $[\alpha]_D^{26}$ -13.5° (*c* 0.74, MeOH) ; FABMS m/z 383 $(M+Na)^+$;IR (film): 3380 , 1705, and 1640 cm^{-1}; ^1H NMR (CD_3OD): δ 6.77 (1H, t, J=7.3 Hz, C_3-H), 5.94 (1H, dd, J=17.8 Hz, 11.0 Hz, C_7-H), 5.28 (1H, dd, J=17.8, 1.2 Hz, C_8-H), 5.21 (1H, dd, J=11.0, 1.2 Hz, C_8-H), 4.36 (1H, d, J= 8.0Hz, $C_{1'}$-H), 3.80 (1H, dd, J=12.8, 2.4 Hz, $C_{6'}$-H), 3.32 (2H, $C_{2'}$- and $C_{4'}$-H, overlapped with solvent signals), 3.17 (1H, t, J= 8.0 Hz, $C_{3'}$-H , 3.16 (1H, m, $C_{5'}$-H), 2.29 (2H, m, C_4-H_2), 1.81(3H, s, C_9-H), 1.71 (2H, dd, J=9.5, 6.6 Hz, C_5-H_2), and 1.40 (3H, s, C_{10}-H).

6) Cronupapine (**2**) as a colorless oil : $[\alpha]_D^{24}$ -55.3° (*c* 3.90, MeOH) ; $C_{24}H_{28}O_{11}Na$ [m/z 515.1530 $(M^+ + Na)^+$]; IR (film): 3400 (br), 1735, and 1615 cm^{-1} ; ^1H NMR (CD_3OD): δ 7.19 (3H, m, C_{15}-, C_{16}- and C_{17}-H), 7.10 (2H, m , C_{14}- and C_{18}-H), 7.07 (2H, d, J= 8.5 Hz, C_2- and C_5-H), 5.04 (1H, t, J=12.7 Hz, C_7-H), 4.90 (1H, $C_{1'}$-H, overlapped with solvent signals), 3.89 (1H, dd, J=12.0, 1.7Hz, $C_{6'}$-H), 3.71 (1H, dd, J=12.0, 4.6 Hz, $C_{6'}$-H), 3.50-3.29 (4H, $C_{2'}$-, $C_{3'}$, $C_{4'}$- and $C_{5'}$-H), 2.98 (1H, d, J=13.7 Hz, C_{12}-H), 2.96 (1H, d, J=17.1 Hz, C_{10}-H), 2.92 (1H, d, J=13.7 Hz, C_{12}-H), 2.59 (1H, d, J=17.1 Hz, C_{10}-H) : ^{13}C NMR (CD_3OD): δ 176.33 (s, C_8), 174.90 (s, C_{11}), 159.97 (s, C_1), 137.43 (s, C_{13}), 132.27 (d, C_3 and C_5), 132.04 (d, C_{14} and C_{18}), 131.62 (s, C_4), 129.84 (d, C_{15} and C_{17}), 128.67 (d, C_{16}), 118.51 (d, C_2 and C_6), 103.01 (s, $C_{1'}$), 78.96 (d, $C_{3'}$), 78.76 (d, $C_{5'}$), 77.87 (d, C_9), 75.68 (d, $C_{2'}$), 72.16 (d, $C_{4'}$), 68.74 (t, C_7), 63.32 (t, $C_{6'}$), 47.07 (t, C_{12}), and 44.87 (t, C_{10}).

7) Under this condition, only the carboxyl group was reduced.

8) The main skeleton of cronupapine may be formed *in vivo*, as shown below.



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