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APORPHINE ALKALOIDS, CD45 PROTEIN TYROSINE PHOSPHATASE INHIBITORS, FROM *ROLLINIA ULEI*

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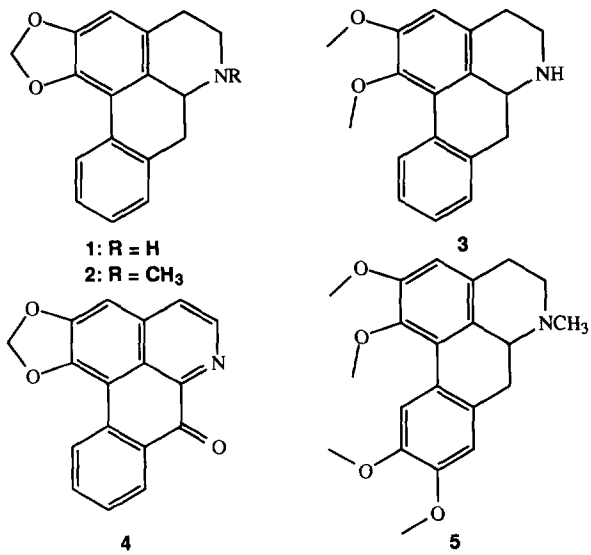
Abstract : Using a high throughput colorimetric bioassay three aporphine alkaloids, annonaine, nornuciferine and roemerine, were isolated and identified as the CD45 protein tyrosine phosphatase inhibitor metabolites of *Rollinia ulei*. The nor-aporphine alkaloids, anonaine (**1**) and nornuciferine (**3**), exhibited more potent inhibitory activity than that of the N-methylated alkaloid roemerin (**2**).

CD45 protein tyrosine phosphatase is a large-molecular-mass transmembrane glycoprotein expressed on all hemaopoietic cells except erythrocytes.[1] Isoforms of this molecule ranging from 180 kDa to 220 kDa are expressed on different cell types[2] and all of them share a common cytoplasmic domain which has tyrosine phosphatase activity.[3-5] Furthermore, the enzymatically active cytoplasmic portion of CD45 alone has been shown to be sufficient for intracellular signaling.[6,7] The importance of CD45 for both T-cell and B-cell receptor signaling has been well documented[8,9] and has generated considerable interest in the study of lymphocyte activation as well as a possible target for drug intervention in various autoimmune and/or inflammatory diseases.

A recent publication describes a sensitive, high throughput, colorimetric assay for screening natural products extracts and chemical compound banks.[10] Using this assay microbial, marine organism and plant extracts were screened for potential CD45 protein tyrosine phosphatase inhibitor secondary metabolites. The dichloromethane extract of the stem and stembark of *Rollinia ulei* (Annonaceae) exhibited 96% inhibition of human CD45 protein tyrosine phosphatase at 30 μg/ml concentration, but showed no inhibition of the non-specific phosphatases alkaline phosphatase and acid phosphatase both human origin or the serine phosphatase calcineurin from bovine brain at 100 μg/ml concentration. Similarly, the dichloromethane extract also inhibited anti-CD3 (T-cell receptor complex) driven IL-2 production in a dose dependent response manner with an IC_{50} of approximately 1 μg/ml. The non-specific stimulation of T-cell IL-2 production by phorbol myristic acetate and ionomycin was not inhibited at this and higher concentrations. These data indicate that the inhibitory components of the plant extract were operating through a T-cell receptor driven response which is known to require a functional CD45 phosphatase.[8,11]



Bioactivity-guided fractionation of the dichloromethane extract of stem and stembark of *R. ulei* yielded three aporphine alkaloids, anonaine (**1**), roemerine (**2**) and nornuciferine (**3**), as the active components of this extract. The structures of these compounds were assigned based on their spectral data and comparison of these data with those reported in the literature.[12-15] CD45 protein tyrosine phosphatase inhibition IC_{50} values for nornuciferine (**3**) anonaine (**1**), and roemerine (**2**) were 5.3 μM, 17 μM and 107 μM, respectively (Figure 1). All compounds were tested in triplicate and the standard error means for nornuciferine, annonaine and roemerine were ± 0.8, 7 and 36, respectively. Liriodenine (**4**) and glaucine (**5**), structurally similar analogs of anonaine (**1**) and roemerine (**2**), were tested for their CD45 protein tyrosine phosphatase inhibitor activity. Glaucine (**5**) displayed similar activity to that of roemerine (**2**) with an IC_{50} value of 88 μM , however, liriodenine exhibited a 182 μM IC_{50} value which was *ca.* ten fold less than that of anonaine (**1**).

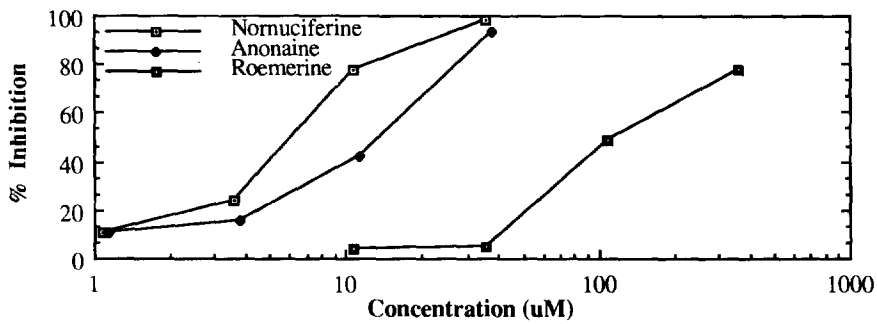


Figure 1. Inhibition of CD45 Protein Tyrosine Phosphatase by the aporphine alkaloids of *Rollinia ulei*.

This is the first report of aporphine alkaloids with CD45 protein tyrosine phosphatase activity, and further biological evaluation of these and related aporphine alkaloids is in progress.

Experimental

PLANT MATERIAL- *Rollinia ulei* was collected in Loreto, Peru and was identified by Prof. Sidney McDaniel, Institute for Botanical Exploration, Mississippi State, Mississippi. Voucher specimens are deposited in the herbarium of Mississippi State University, Mississippi State, Mississippi.

EXTRACTION AND ISOLATION- The air-dried and ground stem and stembark of (550 g) *R. ulei* were extracted with CH_2Cl_2 in a Soxhlett extractor. The solvent was removed on a rotovapor under reduced pressure to yield 5.1g extract. The extract was fractionated on Sephadex LH-20 columns packed in hexane-CH_2Cl_2-MeOH (1:1:1 and 7:4:1) and eluted with same solvent mixture. The resulting active fractions were further separated using aminopropyl-silicagel columns and/or preparative silicagel TLC to yield anonaine (**1**) (1.9 mg), roemerine (**2**) (17.8 mg) and nornuciferine (**3**) (3.5 mg).

BIOLOGICAL ASSAY- Recombinant CD45 phosphatase purified from yeast[16], alkaline phosphatase (human placenta, Sigma®), acid phosphatase (human semen, Sigma®) and calcineurin (bovine brain, Sigma®) were assayed with phosphopeptide substrates using the Malachite Green assay as described previously.[10] Briefly, enzyme, substrate and test article were combined in a Corning Easy-wash microtiter tray in a final volume of 40 µl and incubated for one hour at 37°C. The reaction was terminated with the malachite green reagent and read at 620 nm on a UVmax microtiter plate reader. The tyrosine phosphopeptide FTATEPQ(PO4)YQPGENL was used for assay of CD45, alkaline phosphatase and acid phosphatase at a final concentration of 200 µM. The serine phosphopeptide GRFDRRV(PO4)SVAAE was used to assay calcineurin also at a final concentration of 200 µM. The following buffer systems were used for assay: CD45 phosphatase: 100 mM imidazole, 5 mM DTT, 1 mM EDTA, and 0.05% Tween 20, pH 6.8; alkaline phosphatase: 100 mM glycine, 1 mM ZnCl2, 1 mM MgCl2, pH 10.4; acid phosphatase: 50 mM citrate, pH 4.8; and, calcineurin: 40 mM HEPES, 100 mM NaCl, 6 mM MnCl2, 0.5 mM CaCl2, 0.1 mg/ml bovine serum albumin (BSA), 0.5 mM DTT, pH 7.5. For the acid phosphatase reaction, 80 µL of 0.25 N NaOH was added to each well before addition of the color forming reagent.

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References

1. Trowbridge, I.S.; Ostergaard, H.L.; Johnson, P. *Biochim. Biophys. Acta* **1991**, *1095*, 46.
2. Ralph, S.J.; Thomas, M.L.; Morton, C.C.; Trowbridge, I.S. *EMBO J.* **1987**, *6*, 1251.
3. Charbonneau, H.; Tonks, N.K.; Walsh, K.A.; Fischer, E.H. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 7182.
4. Ostergaard, H.L.; Schakelford, D.A.; Hurley, T.R.; Johnson, P.; Hyman, R.; Sefton, B.M.; Trowbridge, I.S. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 8959.
5. Tonks, N.K.; Diltz, C.D.; Fischer, E.H. *J. Biol. Chem.* **1990**, *265*, 10674.
6. Desai, D.M.; Sap, J.; Schlessinger, J.; Weiss, A. *Cell* **1993**, *73*, 541.
7. Hovis, R.R.; Donovan, J.A.; Musci, M.A.; Motto, D.G.; Goldman, F.D.; Ross, S.E.; Koretzky, G.A. *Science* **1993**, *260*, 544.

8. Koretzky, G.A.; Picus, J.; Schultz, T.; Weiss, A. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 2037.
9. Weaver, C.T.; Pingel, J.T.; Nelson, J.O., Thomas, M.L. *Mol. Cell. Biol.* **1991**, *11*, 4415.
10. Fisher, D.K.; Higgins, T.J. *Pharm. Res.* **1994**, *11*, 759.
11. Pingel, J.T.; Thomas, M.L. *Cell* **1989**, *58*, 1055.
12. Johns, S.R.; Lamberton, J.A.; Sioumis, A.A. *Aust. J. Chem.* **1968**, *21*, 1383.
13. Johns, S.R.; Lamberton, J.A.; Li, C.S.; Sioumis, A.A. *Aust. J. Chem.* **1970**, *23*, 363.
14. Bhakuni, D.S.; Tewari, S.; Dhar, M.M. *Phytochemistry* **1972**, *11*, 1819.
15. Ohashi, M.; Wilson, J.M.; Budzikiewicz, H.; Shamma, M.; Slusarchyk, W.A.; Djerassi, C. *J. Amer. Chem. Soc.* **1963**, *85*, 2807.
16. Pacitti, A.; Stevis, P.; Evans, M.; Trowbridge, I.S.; Higgins, T.J. *Biochim. et Biophys. Acta* **1994**, *1222*, 277.

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