

[PA1-25] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Detection of Nitric Oxide from Rat Platelets

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Griess assays and DAN assay were improved and compared to determine nitric oxide (NO) released from rat platelets. The conventional Griess reaction was improved by replacing the mixture of sulfanilamide and NED ((N-(1-naphthyl) ethylenediamine dihydrochloride) with the separate addition of both. Two Griess assays, the improved conventional and the modified by M. Marzinzig et al which uses dapsone (4,4'-diamino-diphenylsulfone) instead of sulfanilamide, and DAN assay were compared, and DAN assay was suggested to be optimal for the detection of NO formed from rat platelets. For the optimization of DAN assay, assay buffer, platelet count, and deproteinization were considered and Tris (pH 7.5, 0.20M), 2.0*10⁹ platelets/ml, and ultracentrifugation proved to be suitable, respectively. By using this optimized method, NO release from rat platelets were tested against platelet agonists, lipopolysaccharides, antioxidants, etc.

[PA1-26] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Effect of Protostanes from *Alismatis Rhizoma* on Leukotriene C4 in Cellular Systems

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Leukotriene C4 (LTC4) is a potent mediator of asthma and hypersensitivity. This induces bronchoconstriction and increase microvascular permeability. In the present study, the effects four protostane-type triterpens, on arachidonic acid metabolism in cellular systems were examined. They were isolated from ethyl acetate extract of *Alisma Rhizoma* by bioassay-guided isolation using in vitro LTC4 - release assay and identified as alisol B 23-acetate, alisol C 23-acetate, alisol B, and alisol A 24-acetate by spectroscopic methods. The compounds except for alisol C 23-acetate showed significant LTC4 production inhibition activity.

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Inhibition of Tissue Factor by Components from the Fruits of *Chaenomeles Sinensis*

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Tissue factor (TF, tissue thromboplastin, or coagulation factor III) accelerates the blood clotting, activating both the "intrinsic" and "extrinsic" pathways to serve as a cofactor. In order to isolate TF inhibitor from the fruit of *Chaenomeles sinensis*, bioassay-guided purification was carried out to yield seven active compounds, 24-carboxyl-masilnic acid-28-glucopyranoside 2 (IC₅₀=6.0 μg/unit), its aglycone 2a (IC₅₀=2.7 μg/unit), luteolin-7-glucuronide 3 (IC₅₀=18.6 μg/unit), hyperin 4 (IC₅₀=16.6 μg/unit), hoveytrichoside C 7 (IC₅₀=10.4 μg/unit), quercitrin 9 (IC₅₀=81.8 μg/unit) and avicularin 10 (IC₅₀=37.0 μg/unit), when evaluated by one stage clotting assay method. Another compounds such as trachelosperoside A-1 1, apigenin-7-glucuronide methyl ester 5, genistein-7-glucoside 6, luteolin-4'-glucoside 8, (-)-epicatechin 11, luteolin-3'-methoxy-4'-glucoside 12, luteolin-7-glucuronide methyl ester 13 and glucosyl-4'-hydroxy-β ionylidene acetates 14 were inactive in the assay system used. Structures of these fifteen compounds were elucidated by the spectral analysis and chemical method. Compound 1, 7, 14 were isolated for the first time from this plant and compound 2 is a new triterpene.