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Anti-inflammatory chromone alkaloids and glycoside from *Dysoxylum binectariferum* ☆

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ABSTRACT

Herein we report isolation of a new chromone alkaloid chrotacumine K (**12**) from fruits and a chromone glycoside schumaniofioside A (**13**) from leaves of *Dysoxylum binectariferum* Hook f. Schumaniofioside A is reported for the first time from Meliaceae family. Other known alkaloids isolated include rohitukine (**1**) and chrotacumine E (**6**). The structure of new alkaloid **12** was elucidated on the basis of extensive 1D and 2D NMR analysis, synthesis and chemical hydrolysis. Chemically, chrotacumine K (**12**) is a 3′-*O*-acetyl rohitukine which on chemical or enzymatic hydrolysis produces rohitukine. The new alkaloid **12** is also present in seeds and stem-barks of this plant. The glycoside schumaniofioside A (**13**) is present only in leaves, and in abundance (~1% w/w of dried leaves). The isolated compounds and extracts were evaluated for *in vitro* effect on the proinflammatory cytokines (TNF-α and IL-6) in human monocytic THP-1 cells. The alkaloid **12** displayed potent inhibition (57%) of TNF-α at 0.3 μM, and was non-toxic to THP-1 cells up to 40 μM, indicating its excellent therapeutic window. Furthermore, a nitrobenzoyl ester analog **15e** showed better inhibition of IL-6 than parent natural product chrotacumine K.

Dysoxylum species is a rich source of chromone alkaloids. The widely investigated natural product rohitukine (**1**)[1] has been isolated from barks of *Dysoxylum binectariferum* Hook (Meliaceae)[2] and is reported to possess a wide range of biological activities including cytotoxicity,[3] antidyslipidemic,[4] antiadipogenic,[5] gastroprotective,[6] antifertility[7] and antileishmanial activity.[8] Furthermore, this natural product has inspired the discovery of two anticancer clinical candidates flavopiridol[9,10] and P276-00.[11] The structural variations on rohitukine in nature was primarily observed as change in the location of piperidinyl moiety from 8th to 6th position (dysoline)[12] and substitution of piperidine hydroxyl with acyl units, a class of compounds called chrotacumines A–J (**2**–**11**).[13–16] (Fig. 1).

Chrotacumine A and E has modified piperidinyl ring; whereas rest all chrotacumines are 3′-*O*-acyl derivatives of rohitukine. Amongst all 3′-*O*-acyl rohitukine derivatives, most of them are benzoyl derivatives and few are with 3–5 carbon chain containing aliphatic acyl units; however simple 3′-*O*-acetyl-rohitukine has never been reported in the literature.

During our efforts to explore fruits and leaves of *Dysoxylum binectariferum* as an alternative (renewable) source of rohitukine isolation; a new alkaloid chrotacumine K (3′-*O*-acetyl-rohitukine) along with chrotacumine E, schumaniofioside A and rohitukine have been isolated and characterized. Herein, we are reporting for the first time isolation of schumaniofioside A from Meliaceae family. Previously, it was reported from *Pancratium maritimum, Schumanniophyton magnificum* and *Staphylea bumalda* (DNP search). The chemical structures of newly isolated chrotacumine K (**12**), and schumaniofioside A (**13**) are shown in Fig. 2. The bioactivity evaluation of isolated compounds for inhibition of proinflammatory cytokines was also performed.

The HPLC chromatogram of methanolic extract of *Dysoxylum binectariferum* fruits[17] showed three key peaks at t_R 11.5, 12.7 and 17.5 min, respectively (Fig. 3a). The co-TLC with reference standard available in our laboratory indicated that the peak at 11.5 min belongs to rohitukine; however the peak at t_R 12.7 min appeared to be the new one. The methanolic extract was loaded

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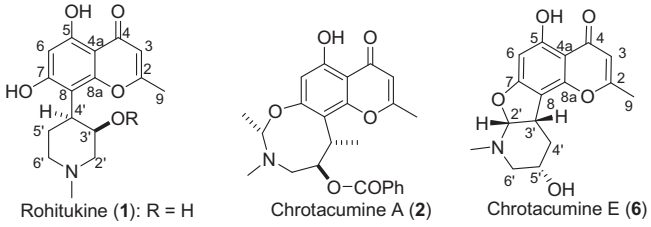

Chrotacumine B (**3**): R = - COC(Me)=CH-Me
Chrotacumine C (**4**): R = -COPh(3,4,5-tri-OMe)
Chrotacumine D (**5**): R = -COPh(3,4-di-OMe)
Chrotacumine F (**7**): R = -CO-CH=CH-Ph
Chrotacumine G (**8**): R = -CO-CH=CH-Ph (3,4,5-tri-OMe)
Chrotacumine H (**9**): R = -CO-CH=C(Me)CH(CH_3)$_2$
Chrotacumine I (**10**): R = -COPh(3,5-di-OMe,4-OH)
Chrotacumine J (**11**): R = -COPh(3-OMe,4-OH)

Fig. 1. Structures of rohitukine (**1**) and chrotacumines A–J (**2**–**11**).

on silica gel column and was eluted with increasing concentration of methanol in chloroform. At 7–8% methanol in chloroform, a new alkaloid was isolated followed by isolation of rohitukine at 12–13% methanol in chloroform. Isolated compounds were characterized by NMR and MS analysis.[18] Rohitukine was characterized by co-TLC with reference standard and by comparison of their spectral data with literature values.[1] The NMR of new alkaloid was recorded in $CDCl_3$ (in order to see phenolic OH signals) as well as in CD_3OD (for comparison with rohitukine).

The alkaloid **12** showed dragendorff positive test, and its ^{1}H NMR spectra was similar (except few differences) to rohitukine, which gave indication that it could be a rohitukine class of compound. In the ^{1}H NMR spectrum of **12**, the signal at δ 4.14 ppm (H-3′) of rohitukine was downfield-shifted to δ 5.18 ppm. Similarly, in the ^{13}C NMR, the signal at δ 66.70 ppm (C-3′) of rohitukine was shifted to δ 71.14 ppm. The ^{13}C NMR also showed the presence of two extra carbons, one at δ 170.6 ppm and other at δ 21.01 ppm. These observations clearly indicated the presence of acetyl group at 3′-OH. The presence of ester group was also confirmed by IR spectra with appearance of stretching vibration at 1737 cm^{-1}. Furthermore, the position of acetyl ester at 3′ position of piperidine ring was ascertained by HMBC studies, wherein a key correlation between H-3′ with C-1″ was observed. The key HMBC correlations of compound **12** are shown in Fig. 2.

It is worth reporting that when compound **12** was submitted for NMR studies in CD_3OD and after checking TLC of the sample after a week, the portion of the **12** was converted to rohitukine (**1**). Furthermore, the HPLC analysis of the methanolic extract of fruits recorded immediately on preparation and after a month indicated that the ratio of rohitukine to chrotacumine K get changed, with significantly increased percentage of rohitukine. The HPLC comparison of rohitukine, chrotacumine K and partially hydrolyzed chrotacumine K is shown in Fig. 3b–d. The ^{1}H and ^{13}C NMR assignments of chrotacumine K (**12**) along with rohitukine and its closely related chrotacumine H (**9**) are shown in Table 1. Further, the presence of chrotacumine K in chloroform and water extracts of fruits of *D. binectariferum* was investigated. Chrotacumine K was present

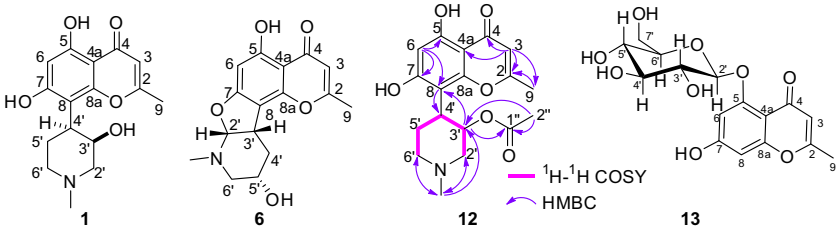

Fig. 2. Chromone alkaloids **1**, **6**, **12** and chromone glycoside **13** isolated from *D. binectariferum*. The key COSY and HMBC correlations of chrotacumine K (**12**) are also shown.

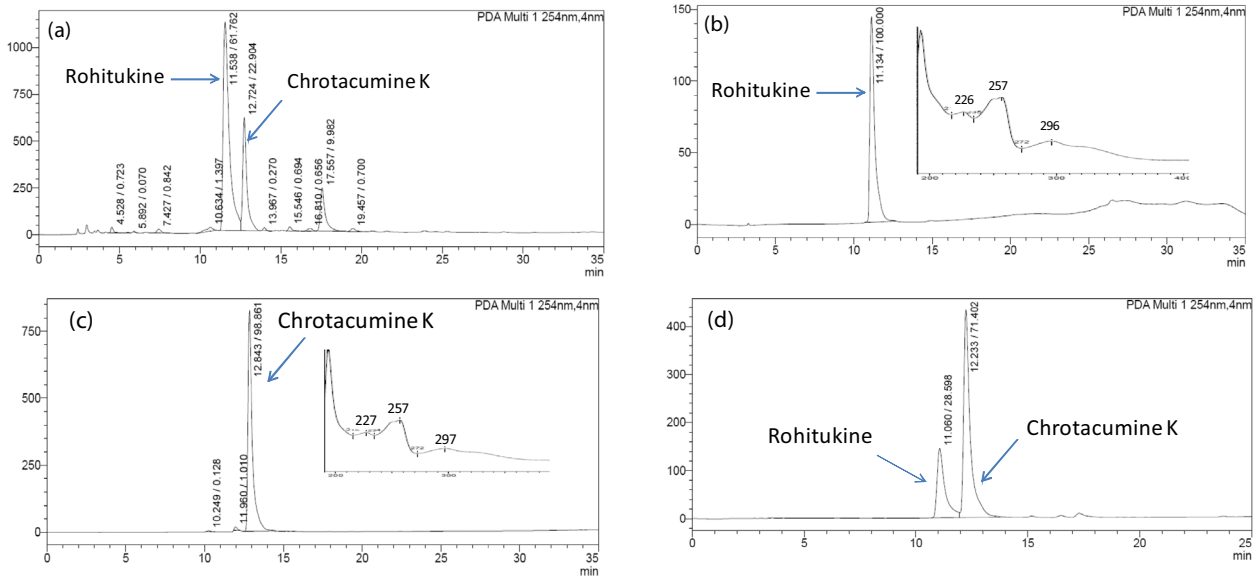

Fig. 3. HPLC chromatogram of (a) fruits of *D. binectariferum* MeOH extract; (b) rohitukine; (c) chrotacumine K; and (d) chrotacumine K hydrolysis to rohitukine in CD_3OD. Insets in Fig. 3b and c are UV chromatograms of respective compounds.