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A NEW ALKALOID FROM FLOWERS OF *ERYTHRINA STRICTA*

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Abstract

The alkaloids present in the flowers of *Erythrina stricta* have been isolated and characterized by spectroscopic methods. A new alkaloid 11-acetyl erysotrine has been isolated and its structure established by spectral studies (UV, IR, ^{1}H NMR and HRMS).

Keywords: *Erythrina stricta*; Leguminosae; Alkaloids; 11-Acetyl erysotrine

Introduction

Erythrina alkaloids occur in species of *Erythrina* (Leguminosae), a genus of wide distribution in tropical parts of the world. These alkaloids possess curare like action. Alkaloidal extracts from different parts of Erythrina species, have been used in indigenous medicine particularly in India [1]. Many pharmacological effects including astringent, sedative, hypotensive, neuromuscular blocking, CNS depressants, laxative and diuretic properties have been also reported for total alkaloidal extract [2-4].

Our interest in phytochemical investigation of extracts of flowers of Erythrina aroused due to the fact that most of the studies have concentrated on examination of seeds which typically Contain 0.1% of alkaloids. Although the alkaloids have been isolated from leaves, stem and barks; no extensive study on alkaloidal content of flowers has been done. Moreover, a preliminary chromatographic investigation of extracts of flowers of *E. stricta* (prickled variety) and *E. indica* (non prickled variety) showed that chloroform extracts of flowers of *E. stricta* have two additional chemical components not present in *E. indica*. We now report findings of our studies on these two chemical components which led to characterization of a new natural product 11-Acetyl erysotrine (A) and another known alkaloid Erythratidinone (B).

Results and Discussion

Crude mixture of alkaloids was obtained by percolation method. The chloroform basic extract was then subjected to preparative TLC which gave two additional spots A and B.

Identification of Compound 'A'

It gave positive Dragon Droff test suggesting that it is an alkaloid. The IR spectrum showed evidence of acetyl carbonyl (1760 cm^{-1}) and no evidence for a hydroxyl group. Its UV absorption at 283.1 nm suggested dioxygenated aromatic ring and absorption at 230.5 nm suggested a diene system. Its Mass

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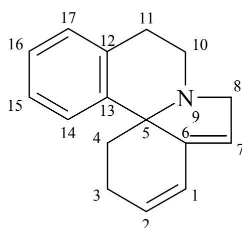
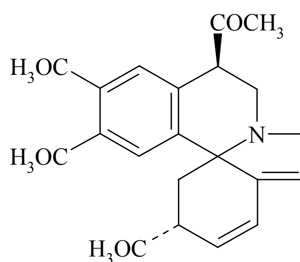
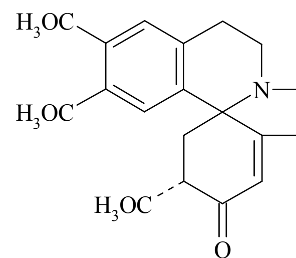


Erythrinan structure (I)



'A' (11-Acetyl erysotrine)



'B' (Erythratidinone)

fragmentation pattern showed molecular ion peak at (M^+, 355) and meta stable ions for the decomposition of M^+ to both (M^+-31) and (M^+-58), suggested that it is an erythrina alkaloid possessing 1,6-diene structure [5].

If an erythrinan carbon skeleton (I) is assumed having a 1-6 diene system with an additional acetyl function in either ring 'C' or 'D', it will be possible to interpret the NMR spectrum completely with the help of the coupling constant values.

Its ^{1}H NMR spectrum indicates the presence of two aryl methoxyl groups at δ3.94 (s) & δ3.85 (s) and an alkyl methoxyl group at δ3.32 (s), two para aromatic protons at δ6.82 (s) & δ6.95 (s) and three vinylic protons at δ6.05 (d, J=10 Hz) δ6.65 (d, J=10 Hz) and δ5.75 (br, s), an acetyl proton signal at δ2.13 (s) and a triplet at δ4.74 corresponding to a proton at C-11.

The two aryl methoxyl groups were assigned to positions C-15 and C-16 respectively so as to explain the two para aromatic protons at C-14 and C-17. Irradiation in the benzylic region causes a sharpening in the signal due to proton at C-17, whereas irradiation of the proton at C-3 produces about 15% Nuclear Overhauser Effect (NOE) for the signal due to the proton at C-14. The NOE effect arises because the proton at C-14 lies over ring A and spatially near the axial C-3 proton. The alkyl methoxyl group is assigned to the C-3 position as in the case of all erythrina alkaloids [6].

The spectrum also indicates an axial proton at C-3 (δ4.05) coupled with the H-4ax (δ 1.87 $J_{3\text{-}4a}$=11.5 Hz), and with the H-4eq (δ2.42 $J_{3\text{-}4e}$=11.5 Hz), the geminal coupling being 11.5 Hz. In addition, H-2 must absorb at δ6.65 and H-1 at δ6.05 ($J_{1,2}$=10 Hz). The methoxyl group at C-3 is therefore equatorial as in other erythrina alkaloids [6].

The assignment of the acetyl group at C-11 was determined by the appearance of a triplet at δ4.74 (J=3.47 Hz) corresponding to a proton attached to a carbon having both phenyl and acetyl substituents. The irradiation of the proton at C-17 caused slight narrowing of the methine signal at δ4.74. The shift of this methine suggested an attached group, resulted in identifying the acetyl group at C-11. The stereochemistry at C-11 was fixed to be 11-β on the basis of a similar coupling constant value as $J_{10,11}$.

The protons at C-8 to some extent are obscured by the O-methyl signals. Moreover, the NMR spectrum of this compound is much more similar to the NMR spectrum of compound erysotrine [6], except for the acetyl peak at δ2.13 and a triplet observed at δ4.74 (H-11). Based on these facts, the new compound was assigned the structure 11-Acetyl erysotrine (A).

The spectral values of compound 'B' was in perfect agreement with the values of a previously known alkaloid Erythratidinone isolated from *E. lithosperma* [6].

Experimental Section

Instrumentation and Conditions

Melting points (°C) were determined on a Fisher-John's melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer spectrophotometer (model 783) the UV absorption spectra were obtained with a Shimadzu UV/VIS spectrophotometer (model 2405) using spectroscopic grade methanol as a solvent.

NMR spectra were recorded on either Varian EM-360 (60 MHz) or Bruker AM (200 MHz) FT-NMR spectrometers. Tetramethylsilane (TMS) was used as an internal standard. The usual employed abbreviations are: s=singlet; br, s=broad singlet; d=doublet, dd= double doublet, t=triplet, m=multiplet, J=coupling constant, δ=chemical shift in ppm, etc. The mass spectra were recorded on a Shimadzu GC-MS, model GP-1000 A.

Chromatographic Methods

Column chromatography

Column chromatography was performed using silica gel G (Acme brand). For broad separations, the ratio between the material to be chromatographed and the

adsorbent was 1:40; while for finer separation was 1:100. The mixture to be loaded on the column was preadsorbed on silica gel. The column was prepared in petroleum ether (60-80%) and was eluted with increasing polarity of solvents starting from petrol to methanol.

Thin layer chromatography

For qualitative work, each plate (10×20 cm) was prepared form a slurry containing silica gel (4 g), distilled water (8 ml) and fluorescent indicator (50 mg). The plates were dried at room temperature and then activated for an hour at 100°C. After developing the plate with a suitable solvent system, it was examined under ultraviolet (254 nm) lamp for detection of the spots. For visualization, plates were exposed to I_2 vapor or sprayed with D.D. reagent (Dragon Droff reagent) or DNP (2,4-dinitro phenyl hydrazine) or with sulphuric acid (10%, heating at 100°C). For quantitative analysis, each preparative TLC plate (20×20 cm) was prepared form a slurry of silica gel (12 g), fluorescent indicator (100 mg) and distilled water (24 ml).

Plant Material

Flowers of *E. stricta* and *E. indica* were collected from the campus of Bhabha Atomic Research Center (BARC). They were identified by Dr. V. Abraham of Nuclear Agriculture Division of BARC.

Extraction of Flowers

Air dried and crushed flowers (100 g) of *E. stricta* and *E. indica* were successively (soxhlet) extracted with petrol, chloroform and methanol. The solvents were removed under pressure to furnish the corresponding crude extracts.

Preliminary TLC studies of crude extracts of *E. stricta* and *E. indica*, indicated that chloroform extract of *E. stricta* had two additional spots (Rf: 0.75 and 0.6 $CHCl_3$) not present in *E. indica*. Therefore, crude chloroform extract of *E. stricta* was further worked up.

Isolation of Crude Alkaloidal Extracts

Alkaloids were extracted by a percolation method. The crude chloroform extract (3.7 gm) was acidified with 0.5 N H_2SO_4 to pH<2. Then the aqueous solution was extracted with ether to remove the neutral compounds. The aqueous extract, after being basified (pH=12) was extracted with chloroform which, on drying under vacuum, yielded the crude mixture of alkaloids (125 mg).

Chloroform basic extracts

The TLC of the alkaloid crude extract revealed the presence of three Dragon Droff positive spots. (Rf=0.8, 0.75, 0.6). Out of these three spots, two were present in *E. stricta* and not in *E. indica* (Rf=0.75 and 0.6). These two spots were separated in pure form by preparative TLC ($CHCl_3$; acetone 95:5 multiple run.), the compounds were named as 'A' (17 mg) and 'B (15 mg)'.

Compound 'A' (11-Acetyl erysotrine) Yield 17 mg (0.017%)

Yellow oil

UV λ_{max}: 230.5 (4.2), 283.1 (3.7)

IR cm^{-1}: 1760 (-COCH3), 1600 (C=C)

^{1}H NMR (200 MHz, $CDCl_3$):

δ1.87 (t, H-4a, $J_{4a,3a}$=11.5 Hz and $J_{4a,4e}$=11.5 Hz), δ2.13 (s, -COCH3 at C-11), δ2.42 (dd, H-4e, J_{4e-4a}=11.5 Hz and J_{4e-3a}=3.5 Hz), δ3.14 (dd, H-10e, $J_{10a,10e}$=13.5 Hz , $J_{10e,11e}$=6.6 Hz), δ3.32 (s, OMe-3), δ3.68 (dd, H-10a, $J_{10a,10e}$=13.5 Hz, $J_{10a,11e}$=3.5Hz)

δ3.85 (s, -OMe-15), δ3.94 s, -OMe-16), δ4.05 (m, H-3a, $J_{3a,4a}$=11.5Hz and $J_{3a,4e}$=3.5Hz), δ4.74 (t, H-11, J=3.4 Hz), δ6.05 (d, H-1, $J_{1,2}$=10 Hz), δ6.65(d, H-2, J=10 Hz)

δ6.82 (s, H-14), δ6.95 (s, H-17).

MS (M^+): 355

Compound 'B' (Erythratidinone) Yield 15 mg (0.015%)

m.p.: 119-120°C

UV λ_{max}: 231 (4.2), 284 (3.6)

IR cm^{-1}: 1675

^{1}H NMR (200 MHz ,$CDCl_3$)

δ2.16 (t, H-4a, $J_{4a,3a}$=13 Hz), δ2.4 to δ3.4 (9H-complex), δ3.49 (s, -OMe-3), δ3.76 (s, OMe-15), δ3.86 (s, OMe-16), δ4.05 (m, H-3a $J_{3a,4a}$=13.2 Hz and J_{3a-4e}=5.4 Hz),δ6.11 (s, H-1), δ6.53 (s, H-14) ,δ6.66 (s, H-17).

MS (M^+): 329

Conclusions

Advent of improved isolation separation methods and powerful analytical techniques have enriched the field of phytochemistry immensely in recent times. Exploration of the blue marine kingdom in addition to the terrestrial greenery has further rejuvenated this field of modern bio-organic chemistry. Truly, the applicative potential of phytochemicals in terms of both their diverse bioactivity and structure archeotype is amazing. The present day natural product research is not confined merely to the isolation and the characterization of compounds but it is based on bio-rational approach. The

case of taxol [7] developing into the wonder anticancer drug is one of the recent examples of this approach. Indeed, a purpose oriented reinvestigation of already examined plants by newer techniques and approaches is likely to pay rich dividends. Therefore, in view of the proven medicinal activity of Erythrina alkaloids, a comparative study of two erythrina species were undertaken in search of bioactive principle which resulted in the isolation and characterization of a new alkaloid 11-Acetylerysotrine, showing insecticidal properties. The entire strategy of structural elucidation and assignment of the stereochemistry was based on non-destructive, NMR spectroscopy and other spectroscopic methods.

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