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# ISOLATION OF AN INSECT ANTIFEEDANT N-METHYLFLINDERSINE AND SEVERAL BENZ[C]PHENANTHRIDINE ALKALOIDS FROM EAST AFRICAN PLANTS; A COMMENT ON CHELERYTHRINE

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Several benzphenanthridine type alkaloids have been isolated by following insect antifeedant assays for fractionation. There is a possibility that the well-known chelerythrine could be an artefact, at least in the present case.

In continuation with our studies on insect antifeedants[1,2] and medicinally active compounds contained in plants, we have investigated the root barks of Fagara chalybea[3], F. holstii (Rutaceae) and Xylocarpus granatum (Meliaceae)[1b,4], all of which taste bitter and are used for medicinal treatment in East Africa[5].

The antifeedant bioassays of the extracts were carried out by the leaf-disk method[1f,1g,6] employing: (i) African armyworms Spodoptera exempta (monophagous) and S. littoralis (polyphagous) on leaves of Zea mays and Ricinus communis respectively, and (ii) the Mexican bean bettle, Epilachna varivestis on leaves of Bountiful stringbeans. This led to the isolation and characterization of N-methylflindersine **1**[7] as the principle responsible for insect antifeedant activity (Table 1). Thus it was found that Zea mays leaves which were dipped in 100 ppm acetone solution of **1** for 2 seconds[8] inhibited the feeding of S. exempta as compared to untreated control leaves; this corresponds to a concentration of ca. 1 μg/cm$^2$.

N-Methylflindersine 1 was also found to be responsible for the antimicrobial activity exhibited by the original 60% aqueous methanol extracts of the root barks. Namely, it was active against the yeast Candida albicans at 50 ppm (minimum inhibitory concentration, MIC) and the fungi Rhizopus delemay at 100 ppm; furthermore, when tested against Trichophyton mentagrophytes it was twice as active as the antibiotic nystatin[9]. However, 1 was only weakly active against gram positive bacteria, e.g., Staphylococcus allreus, Diplococcus pneumoniae and Protens vulgaris, and inactive against gram negative bacteria. In the cytotoxicity test with KB tissue culture, the $ED_{50}$ was 24 μg/ml[10], and according to a modified method[11] of the Ames test[12] employing strain TA-100 of Salmonella typhimurium, 400 revertants per plate could be counted at a concentration of 250 μg/plate in the presence of S-9 mix[13].

During the process of isolation of the active factor, several other known alkaloids of the benz[c]phenanthridine series were also characterized. As shown in Table 1, and Fig.1 these were dihydrochelerythrine[14], chelerythrine[15,16], arnottianamide[17], acetonyldihydrochelerythrine[18], and nitidine[16,19].

It has been reported that chelerythrine and nitidine both exhibit strong antitumor activity but are too toxic for human use[20].

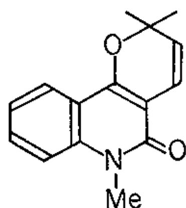
Liquid chromatography was extensively used in all isolations. In the case of F. chalybea constituents, preparative liquid chromatography was applied directly for the first time to the crude hexane extract[3]. Upon standing, a precipitate of crystalline dihydrochelerythrine 2 was obtained in a yield of about 0.1%. Prep LC of the residual 3.5 g (corresponding to ca. 150 g of the dried bark) afforded the following compounds in a total working period of 2 hours: 1 g of a mixture of hydrocarbons, 850 mg of germacrone, 120 mg of a further amount of dihydrochelerythrine 2, 180 mg of a mixture of several compounds, 40 mg of a terpene and 125 mg of N-methylflindersine[3]. In contrast, conventional open column chromatography required 2 weeks using the same amount of the hexane extract, moreover, the unstable dihydrochelerythrine was not isolated by this method probably owing to decomposition while it was left on silica gel column for the

extended period. Arnottianamide 4 and the quaternary ammonium salts, chelerythrine 3 and nitidine 6 were obtained by passage of the chloroform extract through an open silica gel column.

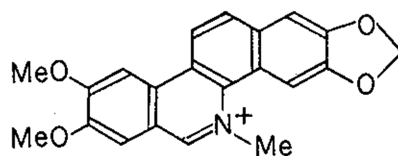
The high-pressure liquid chromatography (hplc) results shown in Fig. 2 are worthy of comments. Namely, when X. granatum was extracted directly with hexane, the extract showed the hplc peak of dihydrochelerythrine 2, (Fig. 2). However, when treated with chloroform, this peak was replaced by that due to chelerythrine 3. In fact dihydrochelerythrine 2 is extremely unstable in solvents like chloroform, methanol and ethanol and is readily converted under normal laboratory conditions into chelerythrine 3 due to the action of air and light: the conversion is much slower, however, in hexane when applied on the plates, the colorless 2 is converted into the colored 3 within several minutes, similarly, the newly chopped bark of F. chalybea is colorless but it turns deep yellow after a few minutes, presumably due to the oxidation of 2 to 3. On the other hand, chelerythrine is readily reduced to the dihydro compound by sodium borohydride. Acetonyldihydrochelerythrine was only seen on the hplc trace when the root bark was extracted with acetone (Fig.2). These results imply that the well documented chelerythrine[15] could be an artefact, acetonyldihydrochelerythrine has already been suggested to be an artefact[18]. As described above, arnottianamide 4 was obtained by passage through silica gel of the chloroform extract of F. chalybea. It was first isolated and characterized by Ishii and coworkers from the bark extract of Xanthoxylum arnottianum and they clearly showed that it could be produced from chelerythrine by oxidation with metaperchloroperbenzoic acid; an elegant oxidation mechanism has also been forwarded[17]. Due to the very facile transformations between 2 and 3, it would not be easy to disprove the presence of chelerythrine in nature, but at least it is safe to conclude that dihydrochelerythrine is by far the major constituent.

The structure of all compounds described in this communication were determined by a combination of spectroscopic methods including NOE measurements in the PMR to determine the substitution patterns of substituents on the aromatic ring.

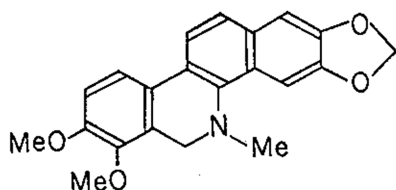
## Figure 1



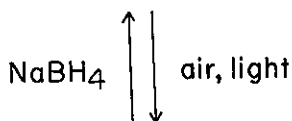

1 N-methylflindersine

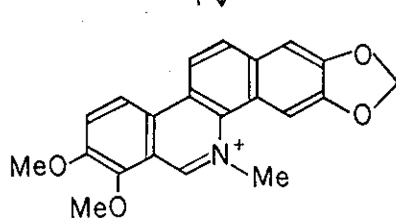



6 nitidine

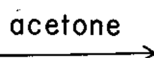


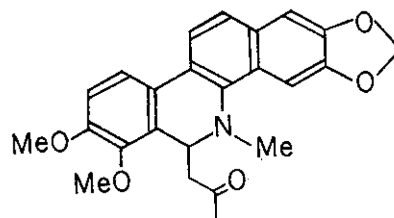

2 dihydrochelerythrine



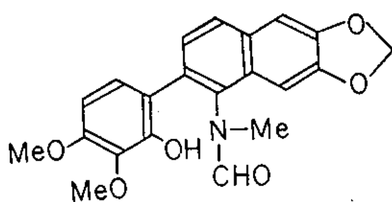




3 chelerythrine




5 acetonyldihydrochelerythrine




4 arnottianamide

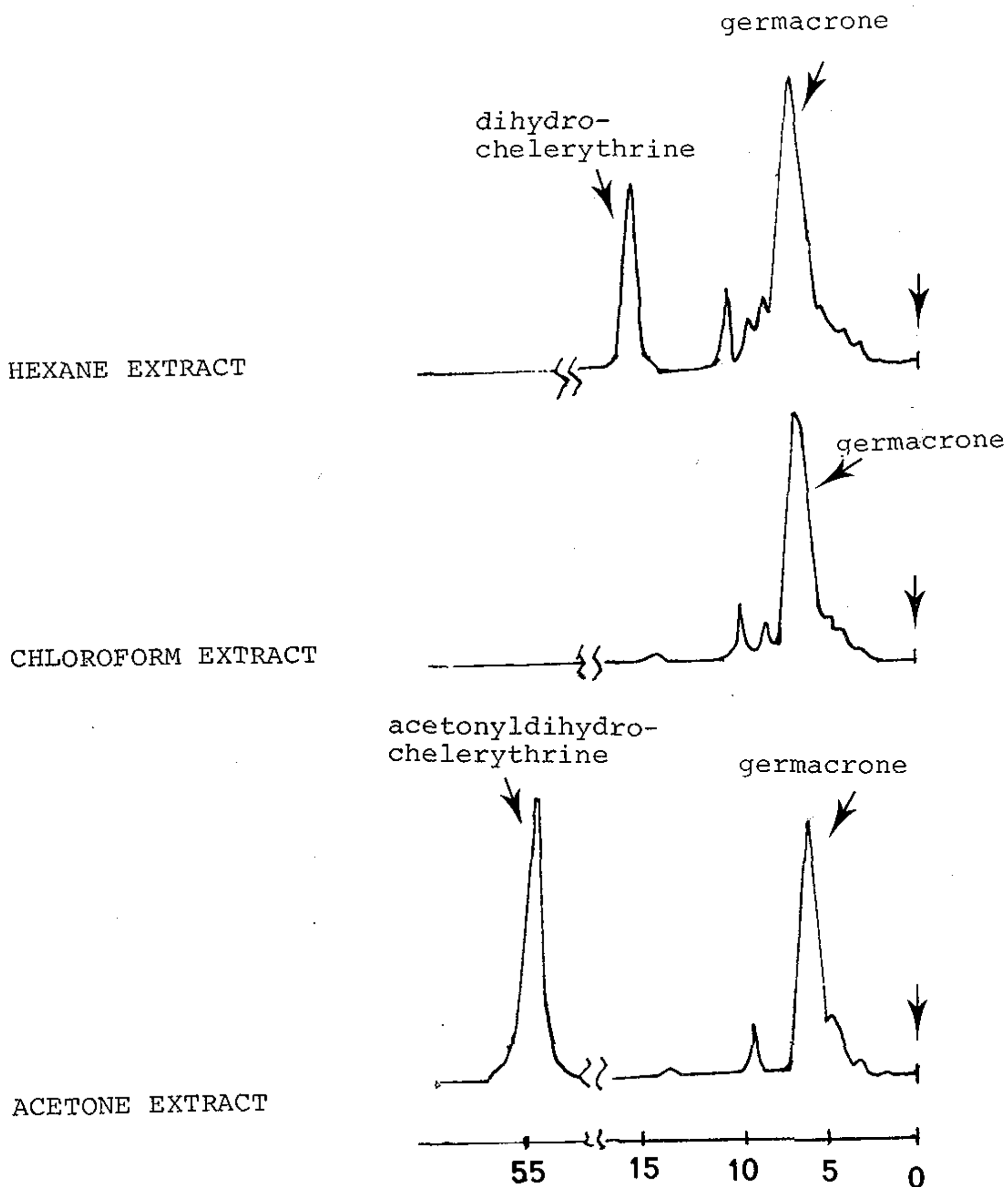



Fig.2. Hplc of X. granatum extracts.
μ-Porasil, 25:75 (ether:n-hexane).

## TABLE 1

| | | *Fagara chalybea* | *F. holstii* | *Xylocarpus granatum* |
|---|---|---|---|---|
| 1 | N-methylflindersine[7] | 0.058%[a] | 0.03 | 0.03[b] |
| 2 | dihydrochelerythrine[14] | 0.18% | 0.09 | - |
| 3 | chelerythrine[15, 16] | 0.0012% | - | - |
| 4 | arnottianamide[17] | 0.006% | - | - |
| 5 | acetonyldihydrochelerythrine[18] | - | - | 0.64 |
| 6 | nitidine[16, 19] | 0.0024% | - | - |

a. Dry weight yield

b. Acetone was used for extraction.

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4) In Ref. 16 we had reported the plant to be Xylocarpus moluscensis but according to information from Prof. D.A.H. Taylor, it should be X.granatum. We are following Prof. Taylor's suggestion, but since the literature is somewhat confused, the correct identity is being checked.

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8) The 2 second restriction in the period of dipping is to avoid extraction and loss of the natural phagostimulants from the leaves.

9) We are grateful to Suntory Co., Ltd., Osaka for this bioassay.

10) We are grateful to Prof.F.J.Schmitz, University of Oklahoma, for having the KB tests run at National Cancer Institute,Md.

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