to give 115 mg of plagiochilide (1); recrystallization from iPr_2O gave colourless needles, mp 106–107° (lit. 110–111°) [1].

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DEHYDROLANUGINOLIDE, A CYTOTOXIC CONSTITUENT FROM THE FRUITS OF *MICHELIA DOLTSOPA**

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Key Word Index—*Michelia doltsopa*; *Michelia excelsa*; Magnoliaceae; 9KB cytotoxicity; germacranolides; lanuginolide; dihydroparthenolide; dehydrolanuginolide.

Abstract—A bioassay-directed isolation scheme yielded three known germacranolides (dihydroparthenolide, lanuginolide and 11,13-dehydrolanuginolide) from an ethanol extract of the fruit of the title plant. Dehydrolanuginolide was identified as the plant constituent responsible for 9KB cytotoxicity.

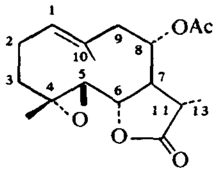
INTRODUCTION

Previous phytochemical work has identified specific germacranolide sesquiterpene lactones and/or alkaloids in the trunk bark [1–5] and leaves and root bark [6] of *Michelia lanuginosa* (Magnoliaceae, Tribe Magnolieae); the roots [5] and trunk bark [7, 8] of *M. champaca*; the trunk bark of *M. cathcartii*; the trunk and root barks [6] of *M. excelsa* (syn.: *M. doltsopa*); and the trunk bark of *M. compressa* [9]. Some of these compounds have exhibited cytotoxicity in the 9KB human nasopharynx carcinoma test system [9]. The discovery of 9KB cytotoxicity in crude extracts of the fruits of *M. doltsopa* prompted our bioassay-directed fractionation of these extracts.

RESULTS AND DISCUSSION

The 9KB activity was concentrated in either $CHCl_3$ or EtOH extracts of the dried fruits. Residue from a large EtOH extract of the defatted fruits was partitioned between $CHCl_3$ and H_2O. The active $CHCl_3$ residue was subjected to repeated column chromatography on Si gel to yield three crystalline compounds (substances A, B and C). A portion of the $CHCl_3$ fraction was extracted with N HCl; the activity remained in the $CHCl_3$ thus eliminating the possible presence of cytotoxic alkaloids.

Two of these compounds (substances A and C) were inactive in the 9KB cytotoxicity assay. Based on physical and spectral properties (mp, ^1H NMR, IR, MS), substances A and C were identified as the respective known germacranolides, lanuginolide (1) and dihydroparthenolide; these identifications were confirmed by TLC comparisons with reference compounds. Substance B was active (ED_{50} 1.8 μg/ml) and chemically seemed somewhat similar to lipiferolide [10]; however, TLC comparisons showed non-identity. Comparisons of ^1H NMR and IR spectra with those of 11,13-dehydrolanuginolide (2) showed a possible identity; reference dehydrolanuginolide was prepared [11] from a small quantity of



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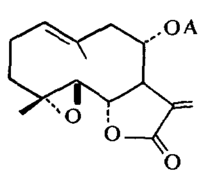

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* Part 9 in the series "Potential Antitumor Agents", For Part 8 see Hembree, J. A., Chang, C.-j., McLaughlin, J. L., Peck, G. and Cassady, J. M. (1979) *J. Nat. Prod.* (*Lloydia*) (in press).

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lanuginolide, and the synthetic and isolated materials were identical (TLC, MS). Dehydrolanuginolide is, thus, another of the cytotoxic α-methylene sesquiterpene lactones originating from natural sources [12].

EXPERIMENTAL

Dried fruits of *Michelia doltsopa* (Buch.-Ham.) ex DC., collected in India, were obtained (B-628976, PR-49483) and authenticated by the Medicinal Plant Resources Laboratory, USDA, Beltsville, Maryland, through which voucher specimens are preserved.

1.592 kg of the ground plant material was defatted (petrol) by Soxhlet extraction. The dried marc was extracted by percolation with EtOH (residue 119 g), and 110 g of the EtOH extract was partitioned between $CHCl_3$–H_2O (1:1). Activity resided in the $CHCl_3$ extract (72.4 g): a 10 g portion of which was partitioned between 100 ml $CHCl_3$ and an equal vol. of N HCl. No activity could be detected in the alkaloid fraction. A 10.99 g sample of the $CHCl_3$ residues was chromatographed on Si gel (50 g), eluted with $CHCl_3$, and the major active fraction (7.155 g) was rechromatographed on 300 g Si gel eluted with C_6H_6; C_6H_6–$CHCl_3$ (1:1): $CHCl_3$ and increments of 0.5–5% MeOH in $CHCl_3$. Based on the observation of homogeneity after TLC and a reaction with $SbCl_3$ indicative of sesquiterpene lactones, a crude white solid (substance A) was isolated after trituration of one of the 1% MeOH in $CHCl_3$ eluants with Et_2O. Monitoring the fractions with the bioassay, 4 fractions, representing 0.5–1% MeOH in $CHCl_3$ eluants, were combined (2.422 g) and rechromatographed on Si gel (150 g) using C_6H_6–$CHCl_3$ (1:1); $CHCl_3$ and increments of 0.2–2% MeOH in $CHCl_3$. Crystallization from Et_2O yielded substance B from the 0.5% MeOH in $CHCl_3$ eluants. Rechromatography on Si gel with $CHCl_3$ and 2% MeOH in $CHCl_3$, of an additional 0.5% MeOH in $CHCl_3$ eluant (270 mg), selected on the basis of TLC monitoring, resulted in the crystallization of substance C.

Lanuginolide (substance A) and dihydroparthenolide (substance C). Recrystallization of substance A from Et_2O produced 59 mg of colourless needles, mp 182–184°, lit. [1] mp 185°. MS, IR and ^{1}H NMR spectra suggested that the compound was lanuginolide, and this identification was confirmed by TLC using reference lanuginolide. Activity in the 9KB system was insignificant (52 μg/ml). Recrystallization of substance C from Et_2O produced 55 mg of colourless needles, mp 135–137°, lit. [1] mp 137°. MS, IR, and ^{1}H NMR spectra aided in the identification of the compound as dihydroparthenolide; TLC with reference material confirmed the identification. No activity was seen (> 100 μg/ml) in the 9KB system.

11,13-Dehydrolanuginolide (substance B). Recrystallization from Et_2O produced 46 mg of colourless needles, mp 167° (d), lit. [2] mp 168° (d). Careful analysis of MS, IR and ^{1}H NMR spectra indicated that substance B was 11,13-dehydrolanuginolide. To substantiate this identification, a small amount of 11,13-dehydrolanuginolide (MS *m/e* 306, M^+) was prepared from 40 mg lanuginolide following the procedure of Takase [11]. The synthesized product and substance B were identical on TLC. Significant activity was exhibited in the 9KB assay (ED_{50} 1.8 μg/ml).

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