injections of FCA but without plant extract. Two weeks after the last injection, the animals were depilated and challenged with serial dilutions in Me_2CO of fractions or pure compounds obtained from chromatography. A 5 μl sample was applied in an 8 mm dia circle by a syringe pipetor. The animals were checked for skin reactions at 24, 48 and 72 hr.

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PLAGIOCHILIDE FROM THE LIVERWORT, *PLAGIOCHILA ASPLENIOIDES*

RÜDIGER MUES*, NOBUO OHNO†, H. D. ZINSMEISTER* and TOM J. MABRY†

* Fachbereich 16—Botanik, Universität des Saarlandes, D-6600 Saarbrücken, W. Germany;
† The Department of Botany, The University of Texas at Austin, Austin, TX 78712, U.S.A.

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Key Word Index—*Plagiochila asplenioides*; Jungermanniales; Hepaticae; plagiochilide; sesquiterpene; ^{1}H NMR; ^{13}C NMR.

A $CHCl_3$ extract of *Plagiochila asplenioides* (L.) Dum. collected in Switzerland afforded a secoaromadendrane-type sesquiterpenoid for which we propose structure **1** on the basis of spectral data. Asakawa *et al.* recently reported the sesquiterpenoid plagiochilide (**1**) from *Plagiochila yokogurensis* [1]. That the compound from *P. asplenioides* corresponds to **1** was confirmed by MS (obs. *m/e* 232.146, calc. 232.146) and by comparison of IR and ^{1}H NMR with those of an authentic specimen. The ^{13}C NMR data also confirm the structure (Fig. 1).

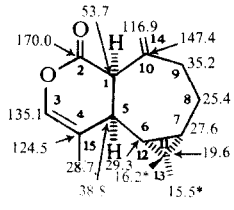

Fig. 1. Plagiochilide (**1**) and its ^{13}C NMR data (ppm, TMS, $CDCl_3$. * Assignment may be changed.

Asakawa *et al.* also recently isolated four novel seco-aromadendrane-type sesquiterpene hemiacetals (plagiochilines C, D, E and F) from a collection of *P. asplenioides* from France [2]; however, they did not detect plagiochilide. The systematic implications of the chemical differences between the Swiss and French specimens of *P. asplenioides* will require studies of collections from other areas.

EXPERIMENTAL

Mps are uncorr. ^{1}H and ^{13}C NMR were measured on HA-100 (Varian) and WH-90 (Bruker), respectively. Si gel 60 (70–230 mesh, Merck) and Si gel 60 GF 254 (Merck) were used for column, TLC and PLC (1.0 mm). Petroleum ether (PE) refers to 30–60° bp range.

Extraction and separation. Plagiochila asplenioides (204 g air-dried material) collected in August, 1977 near Brienz, Bernese Alps, Switzerland (voucher was deposited at the Herbarium of the Fachrichtung Botanik, Universität des Saarlandes, Saarbrücken) was extracted with $CHCl_3$. The solvent was removed *in vacuo* to give 4 g gummy dark syrup. The syrup was chromatographed on a Si gel column (80 g) using a PE–Et_2O gradient elution system. The fraction which eluted with 75% PE and 25% Et_2O was purified further on PLC (PE–Et_2O, 3:1 and 2:1)

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**

JOHN M. CASSADY†, NOBUTOSHI OJIMA, CHING-JER CHANG and JERRY L. MCLAUGHLIN
Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

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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.

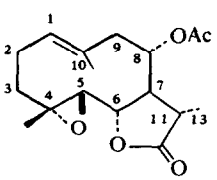
INTRODUCTION

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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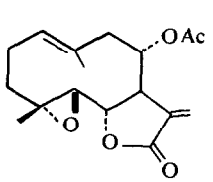

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* Part 9 in the series "Potential Antitumor Agents", For Part 8 see Hembree, J. A., Chang, C.-j., McLaughlin, J. L., Peck, G. and Cassady, J. M. (1979) *J. Nat. Prod.* (*Lloydia*) (in press).

† To whom inquiries should be directed.