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PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF THE CARDIOACTIVE CONSTITUENTS OF THE LEAF OF *DYSOXYLUM LENTICELLARE*

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ABSTRACT.—The cardiac effects of the leaf extract and 11 isolated pure compounds have been examined on isolated, spontaneously beating, atrial muscles of the rat. Using noradrenaline (8×10^{-7}M) and acetylcholine (4×10^{-8}M) as reference drugs and normal saline (equivalent volume) as control, the crude extract of *Dysoxylum lenticellare* (8×10^{-4}g/ml) induced negative chronotropic and positive inotropic responses on the isolated cardiac muscle preparations. The extract demonstrated significant ($p<0.05$-0.01) cardioactivity as attested to by its positive inotropic and/or negative chronotropic activities on the rat atrial preparations. Of the pure isolates tested, **7**, **8**, and **9** demonstrated significant cardiac effects. Although the precise mechanism of action of the extract or pure isolates on the atrial myocardium has not been fully determined, available experimental data suggest that the extract and pure isolates act directly on the cardiac muscle. Alkaloids **7**, **8**, and **9** manifest cardiac effects similar to that of the crude extract but of lesser magnitude. Some indirect effects of these isolates may, however, be associated with the manifested activities.

The genus *Dysoxylum* of the family Meliaceae is composed of about 60 species of trees in Polynesia and Indomalaysia. Phytochemical screening of many species of *Dysoxylum* has indicated that some contain alkaloids (1-3). However, no chemical structures have been determined. We have reported the first structure determinations of alkaloids from the genus, which were isolated from *Dysoxylum lenticellare* Gillespie grown in the Fiji Islands (4). We report here the first bioactivity determinations of the alkaloids and other compounds isolated from the leaf of *D. lenticellare*. Leaves of *D. lenticellare* were extracted with MeOH, and this extract was defatted with pentane from which compounds **1** and **2** were isolated (6). Further partitioning gave a $CHCl_3$ extract containing the known alkaloids **3-9**, an unknown alkaloid, and compound **10** (4-6), which were separated by chromatographic techniques, and the alkaloids were purified, in most cases, as their picrate salts. The 11 compounds isolated from the leaf extract of *D. lenticellare* have been tested for biological activity on the cardiac muscle, along with the crude extract to identify the cardiac constituent(s) of this plant. This paper is the first to report any biological testing on homoerythrina alkaloids and the first of its kind for the 1-phenylethylisoquinoline alkaloid series.

EXPERIMENTAL

PLANT MATERIAL.—*D. lenticellare* was collected by George Uhe on August 28, 1963, near the Boy Scout Camp, Coli-Suva, VitiLevu, in the Fiji Islands. Voucher specimens AK157465 and AK157466 are preserved in the Herbarium of the Auckland Institute and Museum, Auckland 1, New Zealand.

PREPARATION OF PLANT MATERIAL.—The dried plant material (50 g) was extracted with MeOH and the mixture evaporated to dryness. The resulting residue was used in the pharmacological testing.

EXTRACTION.—Powdered leaf material (2.8 kg) was percolated with MeOH. The extract was concentrated to 1.8 liters and partitioned against pentane in a continuous liquid-liquid extractor for several days. The pentane extract after evaporation (96 g) contained no alkaloids (Dragendorff). A 31.9-g portion of this was subjected to column chromatography on Si gel (500 g). The column was eluted with cyclohexane with increasing amounts of EtOAc and MeOH which afforded the isolation of **1** and **2** (6). The brownish, hydroalcoholic layer showed a yellow, solid deposit at the bottom of the flask. This was filtered,

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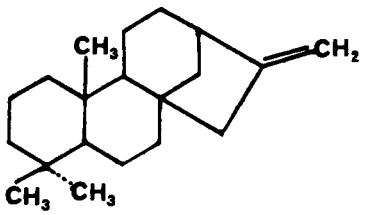

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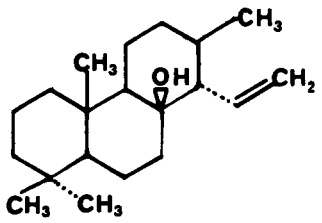

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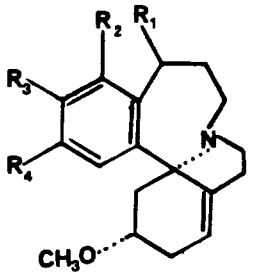
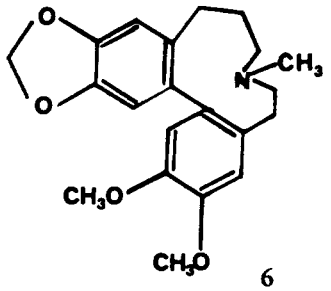


3 R_1=H, R_2=OMe, R_3=R_4=$-OCH_2O-$
4 R_1=H, R_2=H, R_3=R_4=$-OCH_2O-$
5 R_1=H, R_2=H, R_3=R_4=OMe
9 R_1=OH, R_2=H, R_3=R_4=$-OCH_2O-$



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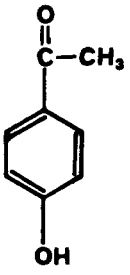

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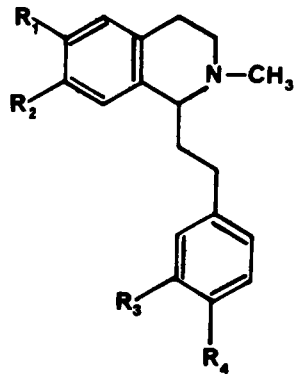

7 R_1=R_2=OMe, R_3=R_4=$-OCH_2O-$
8 R_1=R_2=R_3=R_4=OMe

and 27 g of flavonoid-positive yellow material was removed. The filtrate was concentrated to a syrup. This was diluted with an equal volume of H_2O and was exhaustively extracted with $CHCl_3$. The $CHCl_3$-soluble extract (36 g) was column chromatographed on alumina with increasing amounts of EtOAc in cyclohexane, which afforded the isolation of compounds **3-10** and an unknown alkaloid (4-6). The pure isolates were used for pharmacological investigations.

PHARMACOLOGICAL TESTING.—To prepare spontaneously beating right atria, male and female rats (180-230 g) were killed by stunning and exsanguination. The hearts were quickly exposed, and a piece of cotton thread was tied to the right atrium in situ. The whole heart was then transferred into a petri dish containing Krebs-Henseleit physiological solution (of composition in mM/liter: NaCl, 112.9; KCl, 4.69; $CaCl_2 \cdot 2H_2O$, 2.52; $MgSO_4 \cdot 7H_2O$, 1.18; KH_2PO_4, 1.18; $NaHCO_3$, 25.0 and glucose, 11.0) maintained at room temperature and gassed with carbogen (i.e., 95% O_2+5% CO_2 gas mixture). By means of the attached thread, the right atrium was gently lifted up and separated from the surrounding tissues. Each isolated atrium preparation was then suspended in a 10.0 ml organ-bath containing Krebs-Henseleit physiological salt solution maintained at 37±1° and continuously bubbled with carbogen. The tissue was allowed to equilibrate for a period of 45-60 min (during which the bathing fluid was changed every 15 min until the basal heart rate and amplitude of contractions became steady).

The rate and amplitude of contraction were recorded isometrically by means of an Ugo Basile Force-displacement transducer and a pen-recording microdynanometer (model 7050). The basic frequency of contraction was 248–284/min, and basic force of contraction not less than 0.25 g tension was assumed reasonable to start with (calibration 60 mm = 1.0 g). Graded concentrations of distilled H_2O-dissolved crude extract and pure compounds (in molar concentration) were administered, and the effects of each were recorded.

Sensitivity of the atrial muscle was ascertained at regular intervals with noradrenaline (8×10^{-7}M) and/or acetylcholine (4×10^{-8}M) solutions. The responses are presented as mean values ± SEM, and the level of significance was determined using the Student *t*-test ($n>4$).

RESULTS AND DISCUSSION

The crude extract of the leaf of *D. lenticellare* (8×10^{-4}g/ml) produced a pronounced inhibitory chronotropic effect (-24.18 ± 1.1 change from the control) and an excitatory effect on the amplitude of contraction on the rat atrial muscle preparation ($+10.34\pm2.6$). Because the crude extract possibly contains more than one active constituent and many impurities and still gives such a promising result, the pure isolated and characterized compounds of the leaf were expected to produce better and more promising results.

Out of the 11 isolated compounds, two [**1**,**6**] were insoluble in distilled H_2O and, thus, excluded from the investigation because no suitable solvent was available. The remaining 9, evaluated for cardioactive properties, gave interesting results. While one alkaloid has not been fully characterized, three characterized, isolated compounds, **7**, **8**, and **9**, demonstrated significant ($p<0.05$) cardioactive properties. The chronotropic effect of the crude extract was more prounounced than that of alkaloids **7**, **8** and **9**, but all the pure alkaloids produced a more pronounced effect on the amplitude of contraction than the crude extract (Table 1). The data show that the manifested inhibitory chronotropic effect remains about constant for the alkaloids **7**, **8**, and **9**. This probably suggests an indirect mechanism of action for these pure compounds on the atrial muscle.

TABLE 1. Effects of Leaf Extract and Constituents of *Dysoxylum lenticellare* on the Rate and Amplitude of Contraction of Rat Spontaneously Beating Right Atrium

Constituent Name	Dose	Change from Control (%)	
		Rate	Amplitude
Crude extract	8×10^{-4}g/ml	-24.18 ± 1.1 ($p<0.001$)	$+10.34\pm2.6$ ($p<0.05$) $n=5$
Phyllocladene [**1**]	insoluble	—	—
β-Hydroxysandaracopimarene [**2**]	$1\text{-}16\times10^{-6}$M 8×10^{-5}M	N.M.E.[a]	N.M.E.
3-*epi*-18-Methoxyschelhammericine [**3**]	$2\text{-}8\times10^{-5}$M	N.M.E.	N.M.E.
3-*epi*-Schelhammericine [**4**]	$1\text{-}8\times10^{-5}$M	N.M.E.	N.M.E.
2,7-Dihydrohomoerysotrine [**5**]	$4\text{-}16\times10^{-5}$M	N.M.E.	N.M.E.
Unknown alkaloid	1×10^{-6} up to 8×10^{-5}M	N.M.E.	N.M.E.
Dysazecine [**6**]	insoluble	—	—
Dysoxyline [**7**]	8×10^{-5}M	-6.4 ± 2.2 ($p<0.05$)	$+11.8\pm3.3$ ($p<0.05$) $n=5$
Homolaudanosine [**8**]	8×10^{-5}M	-6.7 ± 1.8 ($p<0.05$)	$+16.3\pm3.0$ ($p<0.01$) $n=6$
3-*epi*-12-Hydroxyschelhammericine [**9**]	8×10^{-5}M	-6.0 ± 2.1 ($p<0.05$)	$+11.5\pm1.6$ ($p<0.01$) $n=4$
p-Hydroxyacetophenone [**10**]	$2\text{-}8\times10^{-5}$M	N.M.E.	N.M.E.

[a]N.M.E. = No manifested effect.

The remaining six isolates gave no effect or produced effects that were not significant when compared with those produced by the control (equivalent volume of normal saline). From these results, it could be rightly said that the cardiac activity of the crude extract of *D. lenticellare* leaf is probably a cumulative effect of the three active constituents **7**, **8**, and **9** together with some contribution by the inactive alkaloids and plant impurities. Alkaloid **8**, homolaudanosine, a 1-phenylethyltetrahydroisoquinoline, probably showed activity due to its moiety and the full methoxylation, which may account for the lesser activity of dysoxyline [**7**]. The activity of 3-*epi*-12-hydroxyschelhammericine [**9**] may be due to the presence of the hydroxyl group, as this is the only alkaloid with a hydroxyl group isolated to date from this plant. Further work is much needed on the two isolates, **1** and **6**, that so far have not been investigated because of their poor H_2O solubility in this effort to identify the cardioactive principle(s) of the extract. These can then be subjected to further pharmacological and toxicological investigations in the development of more potent and less toxic drugs.

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