Antitumor Agents XXXVIII: Isolation and Structural Elucidation of Novel Germacranolides and Triterpenes from *Elephantopus mollis*

KUO-HSIUNG LEE *x, TOSHIRO IBUKA *, HIROSHI FURUKAWA *, MUTSUO KOZUKA *, RONG-YANG WU *, IRIS H. HALL *, and HUAN-CHANG HUANG ‡

Received April 4, 1979, from the **Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514,* and the ‡*School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.* Accepted for publication July 27, 1979.

Abstract □ The ethanolic extract of *Elephantopus mollis* yielded three novel cytotoxic antitumor germacranolides, molephantin, molephantinin, and phantomolin. The extract also yielded three inactive known triterpenes, β-amyrin acetate, lupeol acetate, and epifriedelanol, as well as stigmasterol. The structure and stereochemistry of the cytotoxic antitumor agents molephantin, molephantinin, and phantomolin were determined on the basis of chemical transformations and spectral evidence. Preliminary *in vivo* tumor assays indicated that molephantinin and phantomolin were potent inhibitors of Ehrlich ascites carcinoma and Walker 256 carcinosarcoma. Molephantinin also showed significant antileukemic activity in the P-388 lymphocytic leukemia screen.

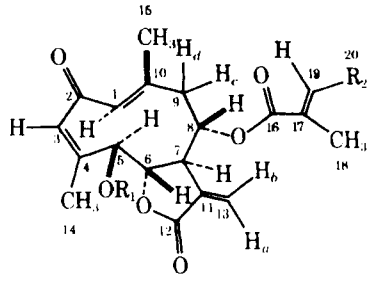
Keyphrases □ *Elephantopus mollis*—isolation and structural determination of molephantin, molephantinin, and phantomolin □ Antineoplastic agents, potential—molephantin, molephantinin, and phantomolin, isolation from *Elephantopus mollis*, structural determination □ Molephantin—isolation from *Elephantopus mollis* and structural determination □ Molephantinin—isolation from *Elephantopus mollis* and structural determination □ Phantomolin—isolation from *Elephantopus mollis* and structural determination

During a continuing search of Formosan plants for agents with potential antitumor activity (1), the alcoholic extract of *Elephantopus mollis*[1] (Compositae) showed significant reproducible inhibitory activity in both *in vitro* (KB cell culture) and *in vivo* (Walker 256 carcinosarcoma) screens[2]. Preliminary reports described the structural determination of three novel cytotoxic antitumor germacranolides, molephantin (I) (4), molephantinin (II) (5), and phantomolin (III) (6). The present report fully describes the isolation and structural elucidation of these novel antitumor sesquiterpene lactones as well as the companion triterpenes β-amyrin acetate (IV), lupeol acetate (V), epifriedelanol (VI), and stigmasterol (VII).

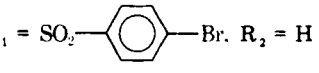
RESULTS AND DISCUSSION

The active alcoholic extract of the whole plant (winter growth) was concentrated and partitioned between water and chloroform. Guided by the assay in KB cells (Scheme I and Table I), the active principles were concentrated in the chloroform extract (Fraction C). Partitioning of this extract between 33% aqueous methanol and hexane concentrated the activity in the aqueous methanolic layer (Fraction D). The aqueous methanolic layer was concentrated and extracted further with chloroform. Chromatography of the active chloroform extract (Fraction G) over silica gel isolated the active principles, molephantin (I), molephantinin (II), and phantomolin (III). Compounds I and III were isolated at 0.025 and 0.023%, respectively, as the major components in this winter collection. In a spring collection of the same plant, I was isolated at ~0.020% as the major cytotoxic principle.

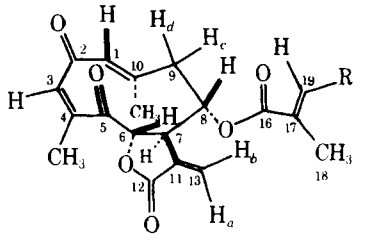
Compound I, mp 212–213°, $C_{19}H_{22}O_6$ (mass spectrum and elemental analysis), contained an α-methylene-γ-lactone, as evidenced by the characteristic NMR signals (Table II) for the methylene protons [δ 6.36 (d, J = 2.5, H_a-13) and 5.83 (d, J = 2.0, H_b-13)] and the IR bands [1773 (γ-lactone) and 1650 (C=C) cm^{-1}]. The UV spectrum of I showed, in addition to the α-methylene-γ-lactone at 210 nm (ε 30,960), a dienone system at 242 nm (ε 18,210, shoulder). The presence of an α,β-unsaturated ester group in I was indicated by IR absorption at 1713 and 1650 (C=C) cm^{-1} and was supported by the base peak at *m/e* 69 [CH_2=$C(CH_3)CO^+$] in the mass spectrum. The NMR spectrum of I suggested a methacrylate pattern for the acid portion of the ester since it showed two multiplets at δ 6.15 and 5.68 (2H, H-19) and one vinyl methyl multiplet at δ 1.96 (H-18)[3], which are typical signals for the methacrylate group (8, 9). Thus, I is an α-methylene-γ-lactone sesqui-



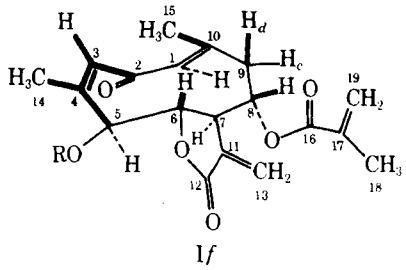
I: R_1 = R_2 = H
Ia: R_1 = $COCH_3$, R_2 = H



Ie: R_1 = SO_2—(p-Br-C$_6$H$_4$)—, R_2 = H



Ib: R = H
IIb: R = CH_3



If

[1] *E. mollis* is known as "Péh-Teng-Khiā-U" in Formosan folklore as an herbal remedy for human rheumatism as well as an antipyretic and anti-inflammatory agent (2).

[2] *In vitro* and *in vivo* assays were performed according to literature procedures (3).

[3] The assignment of δ 1.96 to H-18 was confirmed by the double-resonance experiment carried out on molephantin acetate (Ia).

0022-3549/80/0900-1050$01.00/0

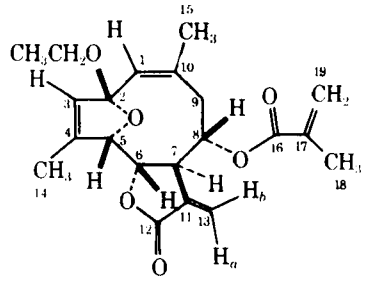
Table I—*In Vitro* Cytotoxicity and *In Vivo* Antitumor Activity of Fractions and Constituents from *E. mollis*

Fraction Constituent	ED_{50} (KB)[a], μg/ml	*In Vivo* Antitumor Activity ($n = 6$)[b]			
		Ehrlich Ascites Carcinoma, % inhibition (33.3 mg/kg)	Walker 256 Carcinosarcoma, T/C (2.5 mg/kg)	Lewis Lung, T/C (25 mg/kg)	P-388 Lymphocytic Leukemia, T/C (25 mg/kg)
A	5.35	—	—	—	—
B	>20	—	—	—	—
C	<20	—	—	—	—
D	<11.50	—	—	—	—
E	>15	—	—	—	—
F	12.50	—	—	—	—
G	3.60	—	—	—	—
I	1.05	75	149	80	118
II	0.90	88	397	123	146
III	4.03	87	378	123	113
Fluorouracil	—	—	—	—	186
Melphalan	—	96	317	—	168
Cyclophosphamide	—	—	—	140	—
0.05% Polysorbate 80	—	0	100	100	100

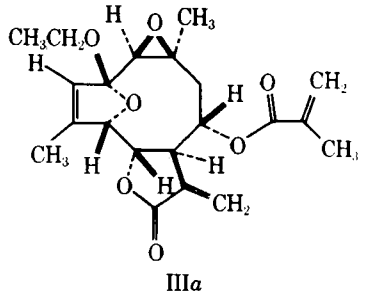
[a] KB refers to human carcinoma of the nasopharynx. Cytotoxicity was assayed according to a literature method (3). A compound is active if the average ED_{50} is ≤4 μg/ml for the pure compound. [b] n = number of animals per group. These *in vivo* data were reported previously (7).

terpene lactone that possesses an additional dienone system and a methacrylate ester.

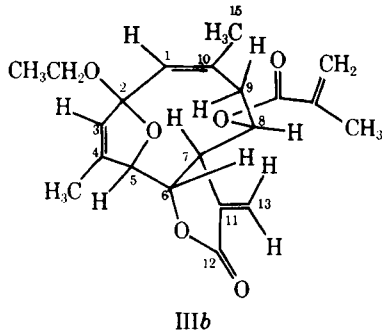
Compound I also possesses a secondary hydroxyl group. It showed an IR absorption band at 3420 cm^{-1} and a M − 18 (water) peak at *m/e* 328.1305[4] in the mass spectrum. The C*H*OH proton (*i.e.*, H-5) was seen as a one-proton singlet at δ 5.46 and was displaced to δ 6.57 in the NMR spectrum of molephantin acetate [I*a*, mp 169°, *m/e* 388.1522 (M^+) for $C_{21}H_{24}O_7$], obtained by acetylation of I with acetic anhydride in pyridine. Since the lactonic proton at C-6 was seen as a doublet (J = 3.5 Hz each) at δ 4.26 in I and 4.35 in I*a*, it indicated that there was almost no coupling between H-5 and H-6 (*i.e.*, the dihedral angle between H-5 and H-6 is ~90°) and that C-4 should be tetrasubstituted, *i.e.*, a vinyl methyl or a carbonyl group was attached at C-4 in I or I*a*.



III



III*a*



III*b*

The assignment of protons at C-7, C-8, C-9, and C-1, as well as the three vinyl methyl groups at C-17, C-10, and C-4, was made possible by spin-decoupling experiments of I*a*. Thus, irradiation at the frequency of H-7 (multiplet) at δ 3.42 collapsed the two doublets of H_a-13 and H_b-13 at δ 6.35 and 5.79 to two singlets, the doublet of H-6 ($J_{5,6}$ = 0 and $J_{6,7}$ = 3.5 Hz) at δ 4.35 to a singlet, and the doublet of triplets of H-8 ($J_{7,8}$ = 10.5 Hz, $J_{8,d}$ = 4.0 Hz, and $J_{8,c}$ = 0) to a pair of doublets ($J_{8,d}$ = 4.0 Hz and $J_{c,d}$ = 11.0 Hz). Conversely, irradiation of H-8 sharpened the multiplet of H-7 as well as converted the doublet ($J_{c,d}$ = 11.0 Hz) of H_c-9 at 2.58 and the doublet of doublets ($J_{c,d}$ = 11.0 Hz and $J_{8,d}$ = 4.0 Hz) of H_d-9 at 2.79 to a pair of doublets ($J_{c,d}$ = 11.0 Hz), thereby suggesting a tetrasubstitution at C-10.

Since most sesquiterpene lactones, especially the germacranolides, contain methyl groups at C-4 and C-10, the two vinyl methyl groups at δ 1.98 (d, J = 1.5 Hz) and 1.80 (d, J < 1.0 Hz) were assigned to these C-4 and C-10 positions, respectively. Consequently, the last oxygen function was placed at C-2, the last position available, as the dienone carbonyl group. This assignment was confirmed by the fact that H-1 and H-3 appeared as a low-field singlet and multiplet at δ 6.37 and 6.05, respectively. Irradiation of the C-4 methyl group at δ 1.98 collapsed the H-3 multiplet to a singlet. Irradiation of the C-17 methyl group at δ 1.94 changed the two one-proton multiplets of H-19 at δ 6.14 and 5.67 to two doublets (J = 1.8 Hz each), confirming the presence of the methylacrylate ester side chain. The remaining narrowly split three-proton doublet at δ 1.79 then was assigned to the vinyl methyl group at C-10.

The foregoing evidence led to the formulation of Structure I for molephantin, exclusive of the complete stereochemistry. A consideration of the relationship between the J value and the dihedral angle (10) with the aid of a Dreiding model established the stereochemical relationships among protons at C-5, C-6, C-7, C-8, and C-9, as shown in I*f*. These assignments were based on the assumption of a β-oriented H-6, followed by the dihedral angles shown between H-5 and H-6, H-6 and H-7, H-7 and H-8, and H-8 and H-9 deduced from their corresponding J values, *i.e.*, $\phi_{5,6} \simeq 90°$ ($J_{5,6}$ = 0), $\phi_{6,7} \simeq 120°$ ($J_{6,7}$ = 3.5 Hz), $\phi_{7,8} \simeq 140°$ ($J_{7,8}$ = 10.5 Hz), $\phi_{8,d} \simeq 50°$ ($J_{8,d}$ = 4.0 Hz), and $\phi_{8,c} \simeq 90°$ ($J_{8,c}$ = 0).

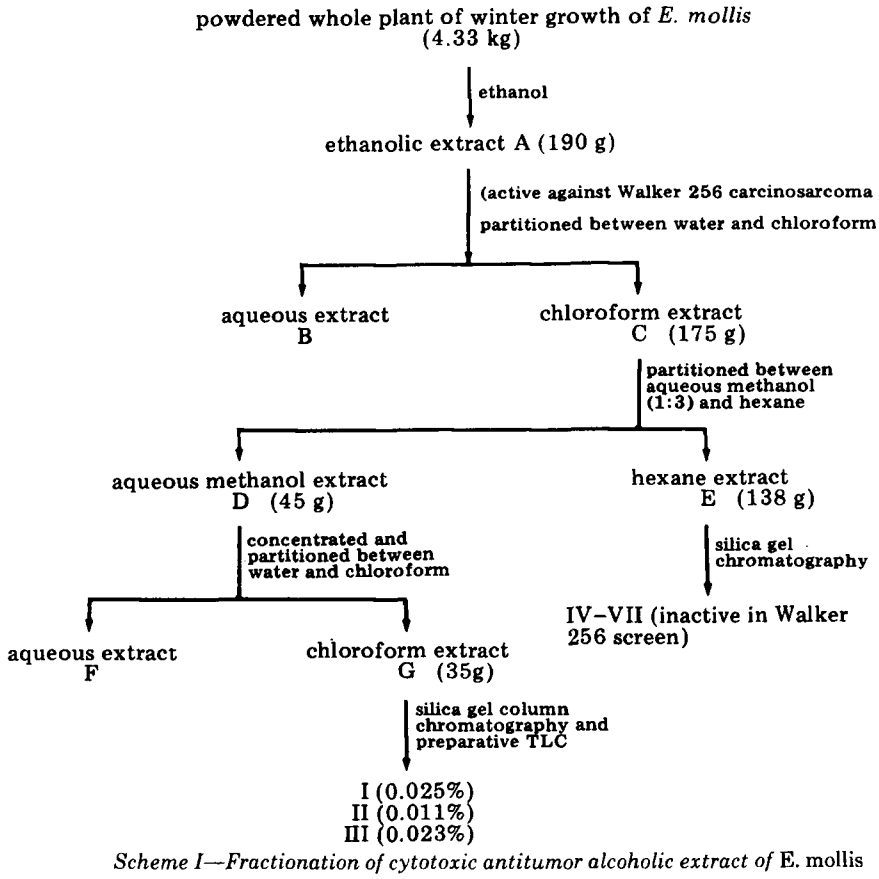
The establishment of these relationships was possible only when the C-10 methyl group was forced to lie close above the π-electron cloud of the C-3–C-4 double bond. This arrangement was supported by the NMR spectra of I and I*a*, in which the C-10 methyl group (δ 1.79 in I and 1.80 in I*a*) was shifted upfield by 0.23 to 0.18 ppm by the C-3–C-4 double bond compared to the C-4 methyl group (δ 2.02 in I and 1.98 in I*a*). A similar upfield shift of H-6 in I and I*a* (δ 4.26 in I and 4.35 in I*a*) further confirmed that H-6 was located close above the C-1–C-10 double bond. When C_5-OH was oxidized to the corresponding ketone [*i.e.*, dehydromolephantin (I*b*), $C_{19}H_{20}O_6$, mp 132–133°, λ_{max} 211 nm (ε 29,070)] by Jones

[4] The calculated *m/e* value for $C_{19}H_{20}O_5$ was 328.1311, which further confirmed the composition of $C_{19}H_{22}O_6$ for I.

Table II—NMR Spectra of I–III and Derivatives[a]

Compound	H-1	H-3	H-5	H-6	H-7	H-8	H_c-9	H_d-9	H_a-13	H_b-13	H-14	H-15	H-18	H-19	H-20	Miscellaneous
I	6.22 (s)	6.01 (br s)	5.46 (br s)	4.26 (d, 3.5)	3.40 (m)	5.26 (dt, 4.0 and 10.5)	2.56 (d, 11.0)	2.78 (dd, 11.0 and 4.0)	6.36 (d, 2.5)	5.83 (d, 2.0)	2.02 (d, 1.5)	1.79 (s)	1.96 (m)	6.15 (m), 5.68 (m)	—	—
I*a*	6.37 (s)	6.05 (m)	6.57 (s)	4.35 (d, 3.5)	3.42 (m)	5.21 (dt, 4.0 and 10.5)	2.58 (d, 11.0)	2.79 (dd, 11.0 and 4.0)	6.35 (d, 2.5)	5.79 (m)	1.98 (d, 1.5)	1.80 (d, <1.0)	1.94 (m)	6.14 (m), 5.67 (m)	1.83 (m)	2.07 (s, OCO-CH_3)
I*b*[b]	5.98 (m)	5.89 (m)[c]	—	4.73 (d, 5.0)	3.97 (m)	5.23 (dt, 4.0 and 10.5)	2.43 (d, 11.0)	2.73 (dd, 11.0 and 4.0)	6.35 (d, 2.5)	5.89 (d, 2.0)	2.21 (d, 2.0)	1.92 (br s)	2.00 (m)	6.15 (m), 5.67 (m)	—	—
I*c*	2.90 (s)	6.09 (m)	—	4.25 (d, 9.0)	4.08 (m)	5.25 (m)	—	—	6.32 (d, 3.0)	5.78 (d, 3.0)	2.31 (d, 2.0)	1.40 (s)	1.98 (m)	6.22 (m), 5.74 (m)[c]	—	3.90 (m, OH)[d], 3.25 (br s, OH)[d]
I*d*	3.46 (s)	6.09 (m)	—	4.23 (d, 9.0)	3.80 (m)	5.10 (m)[c]	—	—	6.28 (d, 3.0)	5.72 (d, 3.0)	2.31 (d, 2.0)	5.21 (m)	1.99 (m)	6.22 (m), 5.71 (m)[c]	—	3.37 (m, OH)[d]
I*e*	6.35 (m)[c]	6.03 (m)	6.49 (s)	4.30 (d, 3.5)	3.35 (m)	6.27 (dt, 4.0 and 10.5)	—	—	6.37 (d, 2.5)	5.85 (d, 2.0)	1.86 (d, 1.5)	1.80 (s)	1.98 (m)	—	—	7.77 (m, 4H, aromatic)
II	6.21 (s)	6.00 (m)	5.46 (br s)	4.25 (d, 3.5)	3.38 (m)	5.24 (dt, 4.0 and 10.5)	2.54 (d, 11.0)	2.76 (dd, 11.0 and 4.0)	6.35 (d, 2.5)	5.82 (d, 2.0)	2.02 (d, 1.5)	1.80 (s)	1.83 (m)	6.92 (br m)	1.83 (m)	—
II*a*	6.36 (s)	6.04 (m)	6.57 (s)	4.34 (d, 3.5)	3.40 (m)	5.21 (dt, 4.0 and 10.5)	2.56 (d, 11.0)	2.76 (dd, 11.0 and 4.0)	6.34 (d, 2.5)	5.77 (m)	1.98 (d, 1.5)	1.80 (d, <1.0)	1.83 (m)	6.92 (br m)	1.83 (m)	2.07 (s, OCO-CH_3)
II*b*	5.94 (m)[c]	6.04 (m)	—	4.77 (d, 5.0)	3.95 (m)	5.32 (dt, 4.0 and 10.5)	2.39 (d, 11.0)	2.72 (dd, 11.0 and 4.0)	6.41 (d, 2.5)	6.04 (d, 2.0)	2.21 (d, 2.0)	1.91 (br s)	1.87 (m)	6.96 (br m)	1.87 (m)	—
III	5.51 (br s)	5.67 (m)	5.23 (4.0)[c]	4.65 (dd, 4.0 and 6.0)	3.14 (m)	5.24 (m)[c]	—	—	6.33 (d, 3.0)	5.80 (d, 3.0)	1.73 (m)	1.79 (d, 1.5)	1.99 (m)	6.17 (m), 5.72 (m)	3.48 (q, 7.0)	1.20 (t, 7.0, H-21)
III*a*[b]	3.05 (s)	5.82 (m)[c]	5.32 (4.0)[c]	4.52 (dd, 4.0 and 6.0)	3.19 (m)[c]	5.23 (m)[c]	—	—	6.45 (d, 3.0)	5.95 (d, 3.0)	1.80 (d, 1.5)	1.41 (s)	2.04 (d, 1.5)	6.24 (m), 5.82 (m)[c]	3.53 (q, 7.0)	1.25 (t, 7.0, H-21)

[a] Run in deuterochloroform at 100 MHz. Values are in parts per million. Multiplicities are indicated by the usual symbols: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet whose center is given; and br, slightly broadened singlet. Figures in parentheses are coupling constants in Hertz. [b] Run in deuterochloroform at 60 MHz. [c] Overlapped signals. [d] Signals disappeared upon addition of deuterium oxide.



Scheme I—Fractionation of cytotoxic antitumor alcoholic extract of E. mollis

reagent, H-6 (δ 4.73 in I*b*) was shifted downfield by 0.38 to 0.47 ppm compared to the ones found in I and I*a*. This shift, coupled with the significant downfield shift of the C-10 methyl group (δ 1.92) in I*b*, suggested that a conformational change had occurred in I*b*.

An examination of the conformation of the 10-membered ring of I*b* based on the dihedral angles derived from the *J* values of H-6 to H-9 in I*b* (Table II) as illustrated in A–C led to the conclusion that Ig was the preferred conformation of dehydromolephantin, in which the C-10 methyl group was in an α-axial configuration and now was deshielded by the C-3–C-4 double bond. The H-1 (δ 5.98) was in a β-axial position and was shielded by the β-oriented C-5 carbonyl group by 0.24–0.39 ppm compared to those found in I (δ 6.22) and I*a* (δ 6.37). The C-1–C-10 double bond was in proximity to the β-oriented C-5 carbonyl group, and this arrangement gave rise to the intramolecular cyclization products when I*b* was treated with sulfuric acid in acetone. These products, which were formed *via* a mechanism shown in Scheme II, were characterized as two guaianolides, Ic and I*d*.

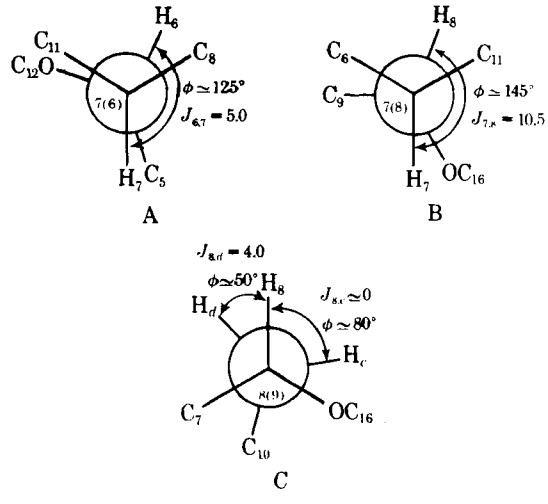
Compound Ic, $C_{19}H_{22}O_7$, mp 168–170°, showed IR (mineral oil) bands at 1750, 1640 (α-methylene-γ-lactone), and 1715 and 1700 (two α,β-unsaturated carbonyl groups) cm^{-1}. The presence of two hydroxyl groups was revealed by IR bands at 3440 and 3320 cm^{-1}, the disappearance of two signals at δ 3.90 (1H) and 2.90 (s, 1H) in the NMR spectrum on the addition of deuterated water, and by an ion of *m/e* 344.1265[5] (M − 18) in the mass spectrum. The NMR spectrum of Ic disclosed the presence of three methyl groups. The three-proton doublet (J = 2.0 Hz) at δ 2.31 was assigned to the cyclopentenone β-methyl group at C-4. The multiplet at δ 1.98 was attributable to the methacrylate methyl group at C-17 as seen in I, I*a*, and I*b*. The methyl singlet in the higher field at δ 1.40 was assigned to the methyl group attached to the carbon bearing a hydroxyl group at C-10. The lactonic proton at C-6 appeared as a doublet with a larger coupling constant compared to that found in I, I*a*, and I*b* and indicated the presence of a second hydroxyl group at C-5 in a guaianolide structure. Assignment of other protons (Table II) was achieved by extensive decoupling experiments. For example, the multiplets at δ 6.22 and 5.74 were assigned to the protons at C-19 since, upon irradiation of the C-17 methyl group at δ 1.98, both changed to two doublets (J = 1.5 Hz each). Irradiation of the C-4 methyl group at δ 2.31 collapsed the multiplet of H-3 at δ 6.09 to a singlet.

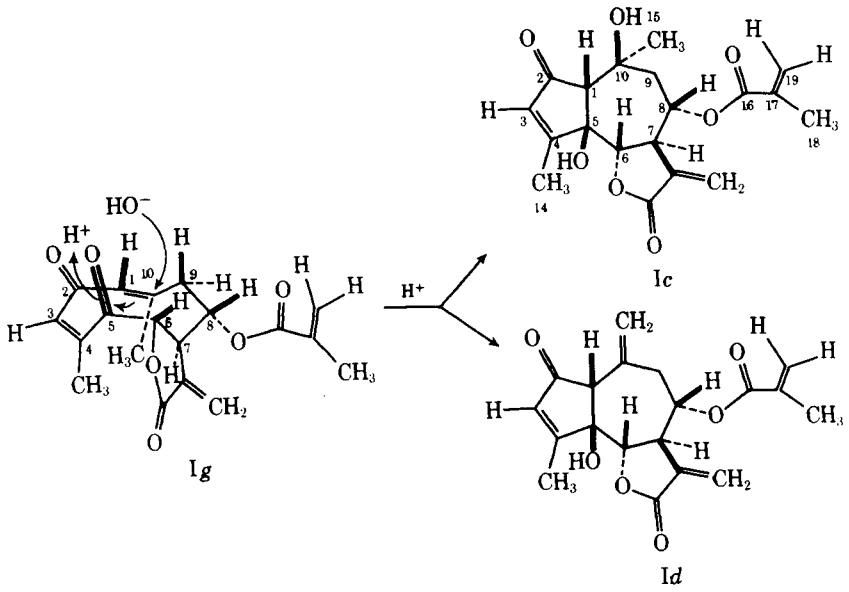
[5] The calculated *m/e* value for $C_{19}H_{20}O_6$ was 344.1260.

Compound I*d*, mp 188–189°, had a composition of $C_{19}H_{20}O_6$ and showed IR (chloroform) bands at 3400, 1770, 1713 (double strength), 1650, and 1625 cm^{-1}. The NMR spectrum of I*d* indicated the absence of the C-10 methyl group as seen in Ic and the presence of one additional terminal methylene group at C-10 at δ 5.21 compared to Ic, suggesting that I*d* was the dehydrated form of Ic. Further assignment of protons (Table II) that supported I*d* as the structure for this product also was achieved by extensive NMR decoupling experiments.

The assignment of a β-hydroxyl and an α-methyl group at C-10 in Ic as well as the β-oriented proton at C-1 and the β-oriented hydroxyl group at C-5 in both Ic and I*d* was based on the mechanism involved in the intramolecular cyclization of I*b* to Ic and I*d*, in which the hydroxyl ion could only make a nucleophilic attack at C-10 of a *trans*-C-1–C-10 double bond from the sterically less hindered β-side since the C-5 carbonyl group was β-oriented. Thus, it gave rise to a β-hydrogen and a β-hydroxyl group at C-1 and C-5, respectively.

This evidence led to the establishment of I*b* for dehydromolephantin, I*a* for molephantin acetate, and I for molephantin. Further unequivocal proof of the structure, stereochemistry, and absolute configuration of I





Scheme II

for molephantin was achieved by a single-crystal X-ray analysis of molephantin *p*-bromobenzenesulfonate (Ie, $C_{25}H_{25}BrO_8S$, mp 165–167°) (4).

Molephantinin (II), mp 223–225°, had a composition of $C_{20}H_{24}O_6$ and showed IR, NMR, and mass spectra similar to those of molephantin (I), except for the difference in the acid portion of the ester side chain. The ester moiety in II was identified as a tigloyl group instead of a methacrylate as in I. This assignment was based on the characteristic NMR signals shown in II for the tigloyl group as the broad vinyl multiplets at δ 6.92 (1H, H-19) and the vinyl methyl multiplets at δ 1.83 (6H, H-18 and H-20) (11, 12), as well as a base peak in the mass spectrum at *m/e* 83 due to the cleavage of a tiglic acid ester (13).

Molephantinin yielded an acetate [IIa, mp 131°, $C_{22}H_{26}O_7$ (*m/e* 402.1670, M^+)] upon acetylation with acetic anhydride in pyridine and a dehydro derivative (IIb, mp 136°, $C_{20}H_{22}O_6$) upon oxidation with Jones reagent. The IR, NMR (Table II), and mass spectral data, especially the data obtained from the extensive NMR decoupling experiments[6], of molephantinin, molephantinin acetate, and dehydromolephantinin were in accordance with the assigned structures of II, IIa, and IIb, respectively. The circular dichroism curve of II showed a strong positive Cotton effect at 244 nm, indicating that molephantinin possessed the same stereochemistry and absolute configuration as molephantin. Accordingly, molephantinin acetate and dehydromolephantinin carried exactly the same stereochemistries as those of molephantin acetate (Ia) and dehydromolephantin (Ib), as depicted in IIa and IIb.

Phantomolin (III) was isolated as a colorless oil and had a composition of $C_{21}H_{26}O_6$ on the basis of high-resolution mass spectrometry (M^+ at *m/e* 374.1880). It was an α-methylene-γ-lactone compound [IR (chloroform) bands at 1770 and 1664 cm^{-1}] and contained a methacrylate ester (1719 and 1640 cm^{-1}) side chain. The NMR spectrum (Table II) confirmed these assignments, showing the α-methylene group of the γ-lactone at δ 6.33 (d, J = 3.0 Hz) and 5.80 (d, J = 3.0 Hz) and the methacryl methyl group at C-17 as a multiplet at δ 1.99 and the two protons at C-19 as multiplets at δ 6.17 and 5.72. The assignment of the one-proton signals at δ 5.25 ($J_{5,6}$ = 4.0 Hz) for H-5, 4.65 (dd, $J_{5,6}$ = 4.0 and $J_{6,7}$ = 6.0 Hz) for H-6, 3.14 (m) for H-7, and 5.24 (m) for H-8 was based on decoupling experiments. Thus, irradiation at the frequency of H-7 at δ 3.14 collapsed two doublets for H-13 to two singlets, changed the doublet of doublets at δ 4.65 to a doublet with J = 4.0 Hz, and sharpened the multiplet at δ 5.24 for H-8. Conversely, irradiation at H-6 sharpened the H-7 and H-8 multiplets.

Irradiation at δ 5.25 (H-5) converted H-6 to a doublet with $J_{6,7}$ = 6.0 Hz. The one-proton broad singlet at δ 5.51 was assigned to H-1 since, upon irradiation of the C-10 methyl group (δ 1.79, d, J = 1.5 Hz), it gave rise to a sharp singlet. The remaining low-field one-proton multiplet at δ 5.67 then was assigned to H-3, which coupled with the C-4 methyl group (δ 1.73, m). The characteristic two-proton quartet (J = 7.0 Hz) at δ 3.48 and the three-proton triplet (J = 7.0 Hz) at δ 1.20 were assigned to an *O*-ethyl group[7]. The location of this group at C-2 rather than C-9 was suggested by the fact that no known germacranolide contained oxygenation at C-9. This assignment also was substantiated by a one-proton multiplet at δ 5.24 for H-8, indicating two protons at C-9. Thus, the last oxygen was placed between C-2 and C-5 as an ether linkage, as shown in III.

Consideration of the cooccurrence of phantomolin (III), molephantin (I), and molephantinin (II) suggested the similar *trans*-axial arrangement among protons at C-6, C-7, and C-8. If the configuration of H-6 was β-oriented, as was likely, then H-7 was α and H-8 was β. The coupling constants of $J_{6,7}$ = 6.0 Hz and $J_{5,6}$ = 4.0 Hz were suggestive of the approximate dihedral angles of 38 and 128° for H-6 and H-7 and 51 and 122° for H-5 and H-6. A Dreiding model indicated the feasibility of only one conformation, *i.e.*, with H-5 as β-oriented, CH_3CH_2O as β-oriented and a C-10 vinyl methyl group *cis* to H-1, as shown in IIIb in which the dihedral angles were ~128° for H-6 and H-7 and ~50° for H-5 and H-6.

Further proof of the structure and relative stereochemistry of III was achieved by an X-ray analysis (6) of a *cis*-1,10-epoxide of phantomolin (IIIa), prepared from epoxidation of III with *m*-chloroperbenzoic acid in chloroform.

Fractionation of the inactive hexane extract (Scheme I) led to the isolation of four colorless crystalline substances, IV (mp 239–241°, $C_{32}H_{52}O_2$), V (mp 218°, $C_{32}H_{52}O_2$), VI (mp 285°, $C_{30}H_{50}O$), and VII (mp 170°, $C_{29}H_{48}O_2$), which were identified as triterpenes β-amyrin acetate, lupeol acetate, epifriedelanol, and stigmasterol, respectively (15, 16).

The *in vitro* cytotoxic molephantin, molephantinin, and phantomolin were tested for *in vivo* antitumor activity against four tumor lines (7). As shown in Table I, molephantinin and phantomolin were potent inhibitors of Walker 256 ascites carcinosarcoma in Sprague–Dawley rats at 2.5 mg/kg/day and of Ehrlich ascites tumor in CF_1 male mice at 33.3 mg/kg/day. Molephantinin also showed significant (T/C ≥ 125%) antileukemic activity in the P-388 lymphocytic leukemia screen at T/C = 146% (DBA/2 mice at 25 mg/kg/day). Further studies on the structure–activity relationships and mechanism of action among compounds related to molephantinin and phantomolin are in progress (17).

EXPERIMENTAL[8]

Isolation of Molephantin (I), Molephantinin (II), and Phanto-

[6] Data on NMR decoupling experiments of molephantinin acetate were reported previously (5).

[7] The presence of an ethyl ether is novel and appears to represent the first example in naturally occurring germacranolides and the second instance of the sesquiterpene lactone lactal hymenolide (14).

[8] Unless otherwise specified, melting points were determined on a Thomas-Hoover melting-point apparatus and are uncorrected. Specific rotations were taken on a Perkin-Elmer model 141 polarimeter (1 = 1 dm). IR spectra were recorded with a Perkin-Elmer 257 grating IR spectrometer in chloroform. NMR spectra were determined in deuterochloroform with a Varian XL-100 NMR spectrometer (tetramethylsilane as the internal standard). Mass spectra were determined on an A.E.I. MS-902 instrument at 70 ev using a direct-inlet system. Circular dichroism was measured on a Cary model 60 spectrometer. Silica gel was used for column chromatography (Mallinckrodt CC-7, 200–325 mesh), TLC (Merck silica gel G), and preparative TLC (Merck silica gel GF 254, 2000 μm). Elemental analyses were performed by Atlantic Microlab, Atlanta, Ga.

molin (III) from *E. mollis*—The *E. mollis* (Compositae) used was from a collection made in January 1973 in Chia-Sen, Kaohsiung, Taiwan[9]. The ground, air-dried, whole plant material (4.33 kg) was extracted exhaustively with ethanol. Guided by the bioassay in KB cells (Scheme I and Table I), the final active chloroform extract (35 g, Fraction G) was chromatographed on silica gel[10] (100 mesh, 7 × 32-cm column) with chloroform, chloroform–acetone (1:1), and acetone as the eluting solvents. Fractions of 1 liter each were collected and examined by TLC.

Fractions 1–4 gave traces of residue upon solvent evaporation. Fractions 5–10 yielded 1.44 g of colorless needles, which later proved to be a 1:1 mixture of I and II after a careful NMR analysis, although they showed only a single spot with different solvent systems. Rechromatography of the mother liquor (9 g) after the isolation of the mixture on a silica gel column (3 × 50 cm) in chloroform (1.5 liters) afforded 270 mg of the same mixture as crystalline solids. A final separation of this mixture was achieved by column chromatography on silica gel (4 × 60-cm column) and elution with chloroform to yield 550 mg (0.011%) of pure II from the first 450 ml of the chloroform eluate and 1.26 g (0.025%) of pure I from the second 600 ml of the chloroform eluate. The mother liquors (5 g), after the isolation of pure I and II, were combined and rechromatographed on silica gel (70–230 mesh, 4 × 70-cm column). The chloroform eluates, which showed a new spot different from I and II on TLC, were combined and chromatographed again on silica gel (70–230 mesh, 2.6 × 60-cm column). Evaporation of the solvent resulting from elution with benzene–chloroform (3:2) yielded pure III (1.035 g, 0.023%).

Isolation of I–III from spring growth of the same plant (collected on March 19, 1972 at the same site) using the described procedure gave rise to different yields, *i.e.*, 0.0199% of I, 0.0086% of II, and 0.015% of III.

Molephantin (*I*)—Molephantin was recrystallized from chloroform–ethyl acetate, mp 212–213°; mass spectrum: *m/e* 346 (M^+), 328.1305 (M − 18) ($C_{19}H_{20}O_5$ requires 328.1311), and 69 [base peak, $CH_2{=}C(CH_3)CO^+$]. Table II shows the NMR data.

Anal.—Calc. for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40. Found: C, 66.48; H, 6.67.

Molephantin Acetate (*Ia*)—Molephantin (10 mg) was acetylated with acetic anhydride (0.2 ml) and pyridine (0.2 ml) for 15 hr at room temperature to yield 10 mg of Ia after usual workup and one recrystallization from ether, mp 169°; IR: 1770, 1745, 1720, 1650, and 1620 cm^{-1}; mass spectrum: *m/e* 388.1522 (M^+, $C_{21}H_{24}O_7$ requires 388.1527), 346 [M − 42 ($COCH_3$)], 328 [M − 60 (CH_3COOH)], 319 [M − 69 [$COC(CH_3){=}CH_2$]], 302 [M − 86 [$HOOCC(CH_3){=}CH_2$]], 277 (M − 42 − 69), 260 (M − 42 − 86), 242 (M − 60 − 86), and 69. The NMR data are shown in Table II.

Anal.—Calc. for $C_{21}H_{24}O_7$: C, 64.93; H, 6.23. Found: C, 64.84; H, 6.38.

Dehydromolephantin (*Ib*)—A solution of molephantin (300 mg) in acetone (5 ml) was cooled to 5–10° with stirring and treated with 20 drops of Jones' reagent. After 5 min, methanol (5 ml) was added and the solution was extracted with ether. The ethereal extract was washed with water, dried (anhydrous sodium sulfate), and evaporated *in vacuo* to afford Ib (145 mg) as colorless prisms after one recrystallization from acetone–ether (1:2), mp 132–133°; IR (mineral oil): 1770, 1720, 1705, 1660, and 1640 cm^{-1}; mass spectrum: *m/e* 344.1256 (M^+, $C_{19}H_{20}O_6$ requires 344.1260). The NMR data are shown in Table II.

Treatment of Dehydromolephantin with Sulfuric Acid: Compounds Ic and Id—A mixture of dehydromolephantin (70 mg) in 0.5% sulfuric acid–acetone (5 ml) was stirred for 30 min at room temperature. The mixture was evaporated *in vacuo*, extracted with chloroform, and dried (anhydrous sodium sulfate). The dried chloroform extract was evaporated to furnish a residue showing two spots on TLC. Separation of this residue was achieved by preparative TLC [chloroform–acetone (10:1)] to yield Ic (14 mg) as colorless prisms (from ether) and Id (40 mg) as colorless needles (from ether).

For Ic, the analytical data were: mp 168–170°; IR (chloroform): 3530, 3410, 1772, 1712, 1692, and 1629 cm^{-1}; mass spectrum: *m/e* 362 (M^+) and 344.1265 (M − 18) ($C_{19}H_{20}O_6$ requires 344.1260). The NMR data are given in Table II.

For Id, the analytical data were: mp 188–189°; IR: 3400, 1770, 1713 (double strength), 1650, and 1625 cm^{-1}; mass spectrum: *m/e* 344.1256 (M^+, $C_{19}H_{20}O_6$ requires 344.1260). The NMR data are given in Table II.

Molephantin p-*Bromobenzenesulfonate* (*Ie*)—A solution of molephantin (25 mg) in dry pyridine (0.5 ml) was added with *p*-bromobenzenesulfonyl chloride (35 mg), and the reaction mixture was allowed to stand at 5°. After 12 hr, the mixture was diluted with water and extracted with chloroform, dried, and evaporated to give a product. Recrystallization from isopropanol–ether (1:1) afforded colorless needles, mp 165–167°.

Molephantinin (*II*)—The analytical data for II were: mp 223–225° (ethanol); IR: 3420, 1775, 1713, 1650, and 1615 cm^{-1}; mass spectrum: *m/e* 360 (M^+), 342.1462 (M − 18) ($C_{20}H_{24}O_6$ requires 342.1467), and 83 [base peak, $CH_3CH{=}C(CH_3)C{=}O^+$]. The NMR data are shown in Table II.

Molephantinin Acetate (*IIa*)—Acetylation of molephantinin (10 mg) with acetic anhydride in pyridine in the usual way gave IIa as colorless prisms in quantitative yield, mp 131° (ether); IR: 1775, 1745, 1713, 1655, and 1615 cm^{-1}; mass spectrum: *m/e* 402.1670 (M^+) ($C_{22}H_{26}O_7$ requires 402.1679), 360 [M − 42 ($COCH_3$)], 342 [M − 60 (CH_3COOH)], and 83 [base peak, $CH_3CH{=}C(CH_3)CO^+$]. The NMR data are shown in Table II.

Dehydromolephantinin (*IIb*)—Molephantinin (26 mg) in acetone (2 ml) was oxidized with Jones' reagent (0.3 ml) in the manner described for Ib. The product was purified by preparative TLC [developed with chloroform–acetone (5:1)] and recrystallized from ether to give IIb (24 mg) as colorless pillars, mp 136°; IR: 1776, 1710, 1660, and 1620 cm^{-1}; mass spectrum: *m/e* 400 (M^+). The NMR data are shown in Table II.

Anal.—Calc. for $C_{20}H_{22}O_6$: C, 67.02; Found: C, 67.25: H, 6.23.

Phantomolin (*III*)—Phantomolin was isolated as a colorless oil; IR: 1770, 1725, 1719, 1664, and 1640 cm^{-1}; mass spectrum: *m/e* 374 (M^+). The NMR data are shown in Table II.

Anal.—Calc. for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 67.40; H, 6.98.

Phantomolin cis-*1,10-Epoxide* (*IIIa*)—A solution of phantomolin (15 mg) in chloroform (1 ml) was treated with a solution of *m*-chloroperbenzoic acid (20 mg) in chloroform (1 ml), and the mixture was allowed to stand at room temperature for 48 hr. The chloroform was evaporated under reduced pressure, and the residue was dissolved in ether. The ether solution was washed with 1% sodium bicarbonate and water, dried, and evaporated *in vacuo* to yield a product. This product, upon preparative TLC separation, yielded IIIa as colorless needles (6 mg) after one recrystallization from ether-hexane (3:1), mp 172°; IR: 1775, 1725, 1672, 1660, and 1643 cm^{-1}; mass spectrum: *m/e* 390 (M^+). The NMR data are shown in Table II.

Anal.—Calc. for $C_{21}H_{26}O_7$: C, 64,60; H, 6.71. Found: C, 64.31; H, 6.68.

Isolation and Characterization of β-Amyrin Acetate (IV), Lupeol Acetate (V), Epifriedelanol (VI), and Stigmasterol (VII)—The nonpolar *n*-hexane extract (~80 g) was chromatographed on a silica gel column[10] (silicic acid, 100 mesh, 10 × 100 cm) with *n*-hexane and then *n*-hexane–chloroform (9:1) as the eluents.

The first eluate from *n*-hexane was evaporated to dryness. Recrystallization of the residue from *n*-hexane afforded 400 mg of IV as colorless needles, mp 239–241°, $[\alpha]_D^{20}$ +74° (*c*, 1.0 in chloroform). The IR spectrum of IV was identical to that of an authentic sample of β-amyrin acetate[11] (15).

Anal.—Calc. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.99; H, 11.33.

The second eluate from *n*-hexane was evaporated to dryness. Recrystallization of the residue from *n*-hexane–ether (1:1) afforded V (10 mg) as colorless needles, mp 218°, $[\alpha]_D^{20}$ +40.1° (*c*, 1.0 in chloroform). The IR spectrum (in potassium bromide) of V was identical to that of an authentic sample of lupeol acetate[11].

Anal.—Calc. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.89; H, 11.00.

The first eluate from *n*-hexane–chloroform (9:1) was evaporated to dryness. Recrystallization of the residue from chloroform gave VI (20 mg) as colorless prisms, mp 285°, $[\alpha]_D^{20}$ +21° (*c*, 1.0 in chloroform). The IR spectrum of VI was superimposable with that of an authentic sample of epifriedelanol[11] (16).

Anal.—Calc. for $C_{30}H_{52}O$: C, 84.04; H, 12.33. Found: C, 84.24; H, 12.43.

The second eluate from *n*-hexane–chloroform (9:1) was evaporated to dryness. Recrystallization of the residue from acetone yielded VII (400 mg) as colorless silky needles, mp 170°, $[\alpha]_D^{20}$ −50.0° (*c*, 1.0 in chloroform); mass spectrum: *m/e* 412 (M^+). A mixed melting point of VII with authentic stigmasterol (16) showed no depression, and the IR spectra were identical.

Anal.—Calc. for $C_{29}H_{48}O_2$: C, 84.40; H, 11.72. Found: C, 84.23; H, 11.79.

[9] A voucher specimen is available for inspection at the herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.

[10] Mallinckrodt.

[11] Provided by Dr. T. Kikuchi.

REFERENCES

(1) K. H. Lee, Y. Imakura, Y. Sumida, R. Y. Wu, I. H. Hall, and H. C. Huang, *J. Org. Chem.*, **44**, 2180 (1979).

(2) A. T. Hsieh and T. I. Yang, "Nomenclature of Plants in Taiwan," National Taiwan University Press, Taiwan, Republic of China, 1969, p. 924.

(3) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).

(4) K. H. Lee, H. Furukawa, M. Kozuka, H. C. Huang, P. A. Luhan, and A. T. McPhail, *J. Chem. Soc. Chem. Commun.*, **1973**, 476.

(5) K. H. Lee, T. Ibuka, H. C. Huang, and D. L. Harris, *J. Pharm. Sci.*, **64**, 1077 (1975).

(6) A. T. McPhail, K. D. Onan, K. H. Lee, T. Ibuka, M. Kozuka, T. Shingu, and H. C. Huang, *Tetrahedron Lett.*, **1974**, 2739.

(7) I. H. Hall, K. H. Lee, C. O. Starnes, S. A. ElGebaly, T. Ibuka, Y. S. Wu, T. Kimura, and M. Haruna, *J. Pharm. Sci.*, **67**, 1235 (1978).

(8) F. A. Bovey, "NMR Data Tables for Organic Compounds," vol. 1, Wiley, New York, N.Y., 1967, p. 136.

(9) S. M. Kupchan, A. Aynehchi, J. M. Cassady, H. K. Schnoes, and A. L. Burlingame, *J. Org. Chem.*, **34**, 3869 (1969).

(10) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).

(11) R. R. Fraser, *Can. J. Chem.*, **38**, 549 (1960).

(12) J. D. Connolly, R. Henderson, R. McCrindle, K. H. Overton, and N. S. Bhacca, *J. Chem. Soc.*, **1965**, 6935.

(13) T. A. Geissman and T. S. Griffin, *Rev. Latinoam. Quim.*, **2**, 81 (1971).

(14) W. Herz, K. Aota, M. Holub, and Z. Samek, *J. Org. Chem.*, **35**, 2611 (1970).

(15) T. Kikuchi, T. Yokoi, M. Niwa, and T. Shingu, *Chem. Pharm. Bull.*, **25**, 2078 (1977).

(16) T. Kikuchi, T. Toyoda, M. Arimoto, M. Takayama, and M. Yamano, *Yakugaku Zasshi*, **89**, 1358 (1969).

(17) K. H. Lee, "Program and Abstracts of the 16th National Medicinal Chemistry Symposium," Medicinal Chemistry Division, A ıerican Chemical Soçiety, Kalamazoo, Mich., 1978, pp. 44–58.

ACKNOWLEDGMENTS

Presented in part at the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, Chicago meeting, August 1974.

Supported by National Cancer Institute Grant CA-17625 and American Cancer Society Grant CH-19 to K. H. Lee.

The authors thank Dr. Tohru Kikuchi, Research Institute for Wakan-Yaku (Oriental Medicines), University of Toyama, Toyama, Japan, for providing authentic samples of β-amyrin acetate, lupeol acetate, epifriedelanol, and stigmasterol; Dr. D. L. Harris, Department of Chemistry, University of North Carolina at Chapel Hill, for the 100-MHz NMR spectra; and Dr. David Rosenthal and Mr. Fred Williams, Research Triangle Center for Mass Spectrometry, for mass spectral data.

Convenient Synthesis of (*S*)-(+)-Apomorphine from (*R*)-(−)-Apomorphine

PATRICK J. DAVIS *, SELIM SEYHAN *, WILLIAM SOINE ‡§, and ROBERT V. SMITH ‡x

Received May 7, 1979, from the **Division of Pharmaceutical Chemistry and the* ‡*Drug Dynamics Institute, College of Pharmacy, University of Texas at Austin, Austin, TX 78712.* Accepted for publication June 1, 1979. §Present address: Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, VA 23298.

Abstract □ A method was devised for preparing (*S*)-(+)-apomorphine from (*R*)-(−)-apomorphine. Dehydrogenation of the dimethyl ether of (*R*)-(−)-apomorphine with 10% palladium-on-carbon followed by reduction with sodium cyanoborohydride under acidic conditions resulted in quantitative racemization to give (*R,S*)-apomorphine dimethyl ether, which then was resolved with (−)-tartaric acid. Ether cleavage of (*S*)-(+)-apomorphine dimethyl ether (−)-tartrate with hydriodic acid in acetic anhydride yielded (*S*)-(+)-apomorphine, which was isolated as the hydrochloride salt in 99% enantiomeric excess.

Keyphrases □ Apomorphine—synthesis of (*S*)-(+)-stereoisomer from (*R*)-(−)-stereoisomer □ Dopaminergic agonists—apomorphine, synthesis of (*S*)-(+)-stereoisomer from (*R*)-(−)-stereoisomer □ Stereoisomers—apomorphine, synthesis of (*S*)-(+)-stereoisomer from (*R*)-(−)-stereoisomer

Current interest in apomorphine (I) stems from its activity as a dopaminergic agonist and its consequential antiparkinsonism activity (1–3). Clinical utility has been demonstrated for I alone and in combination with other agents such as levodopa (4). Further studies are aimed at preparing suitable prodrug derivatives to overcome the drawbacks to its use (5). In addition to parkinsonism, recent studies indicated potential new uses for I in ameliorating the symptoms of Huntington's chorea (6), tardive dyskinesia (7), Gilles de la Tourette's syndrome (8), and schizophrenia (9, 10).

BACKGROUND

Most work to date involved the use of (−)-apomorphine (*R*-configuration), synthesized commercially by the acid-catalyzed rearrangement of (−)-morphine (11). Investigations into the pharmacological properties of (*S*)-(+)-apomorphine (II) have been limited to *in vivo* assays including dog emesis, a caudate brain-lesioned mouse preparation (12, 13), and evaluation of the racemate in comparison to (*R*)-(−)-I in these assays as well as on overt behavior in the monkey (13, 14). Previous reports utilized a *de novo* synthesis of (*R,S*)-I *via* Pschorr cyclization followed by resolution of the racemate (12, 15).

Molecular pharmacologists have extended the knowledge of dopaminergic mechanisms by the examination of several receptor assays, culminating in the postulation of several distinct dopamine receptors (16). Along with other criteria, these receptors are distinguished from one another by the differential action of dopaminergic agonists and antagonists, including (*R*)-(−)-I. Thus, apparently difficult to rationalize effects, such as the improvement of schizophrenic symptoms with exceedingly low doses of I, possibly can be understood on the basis of presynaptic dopamine receptors modulating dopaminergic transmission and, consequently, postsynaptic dopaminergic activity (9).

Because of the renewed interest in apomorphine pharmacology and the known differential receptor activity of stereoisomeric pharmacological agents (17), a reexamination of the two antipodes of I was desired. Also, a more direct method was sought for the synthesis of II from the commercially available (*R*)-(−)-antipode rather than the *de novo* synthesis. The synthesis of II reported here is patterned after an earlier procedure for racemization of the related aporphine alkaloid glaucine (18).

EXPERIMENTAL

Reagents—(*R*)-(−)-Apomorphine hydrochloride hemihydrate[1], 10% palladium-on-carbon[2], sodium cyanoborohydride[3], (+)- and (−)-tartaric acid[3], *p*-tolylsulfonylmethylnitrosamide[3], and heptafluorobutyric an-

[1] McFarland Smith Ltd., Edinburg, Scotland.
[2] Alfa-Ventron, Danvers, Mass.
[3] Aldrich Chemical Co., Milwaukee, Wis.

0022-3549/80/0900-1056$01.00/0