Synthesis of [8,9-$^2H_2$]Apomorphine and [1,3,8,9-$^2H_4$]Apomorphine for PMR, ^{13}C-NMR, and Mass Spectral Studies

WILLIAM H. SOINE *, JAMES E. HUDSON ‡, BEN A. SHOULDERS ‡, and ROBERT V. SMITH *x

Received March 23, 1979, from the **Drug Dynamics Institute, College of Pharmacy, and the* ‡*Department of Chemistry, University of Texas at Austin, Austin, TX 78712.* Accepted for publication June 6, 1979.

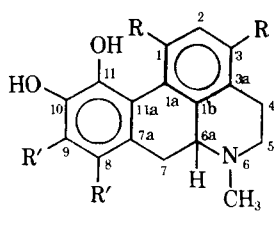
Abstract □ Methods are described for the preparation of [8,9-$^2H_2$]-apomorphine and [1,3,8,9-$^2H_4$]apomorphine based on reaction of apomorphine in trifluoroacetic acid-*d*. The mass spectral properties of these compounds and of the *O,O*-bis(heptafluorobutyrate) ester and the *O,O*-bis(trimethylsilyl) and *O,O*-bis(*tert*-butyldimethylsilyl) ethers of apomorphine, using electron impact and chemical ionization, are reported. [1,3,8,9-$^2H_4$]Apomorphine was used to elaborate the ^{13}C-NMR chemical shifts of the proton-bearing carbons of apomorphine.

Keyphrases □ Apomorphine—synthesis of deuterated analogs for PMR, ^{13}C-NMR, and mass spectral analyses □ Mass spectrometry—analysis, deuterated analogs of apomorphine □ NMR spectroscopy—analysis, deuterated analogs of apomorphine

There is a resurgence of interest in the aporphine alkaloid apomorphine (I) because of its utility in the treatment of Parkinson's disease (1–3), tardive dyskinesia (4), Huntington's chorea (5, 6), and schizophrenia (7). As part of ongoing studies of the mammalian metabolism of I (8–11), methods were desired for the preparation of its specifically deuterated analogs. The latter compounds were envisioned as being potentially useful in mass spectral assays of I and metabolites in biological fluids (12).

Early studies on the deuteration of I gave conflicting evidence on the site and extent of deuteration (13) or provided methodology requiring total synthesis of the aporphine skeleton (14). One report (13) suggested that deuterated analogs of I would not be suitable for use in a mass spectral assay due to proton scrambling during vaporization. Therefore, mass spectral studies were initiated with deuterated I to develop conditions suitable for use in biological assays.

During preliminary analysis, PMR analysis of I in trifluoroacetic acid-*d* showed facile exchange of the 8- and 9-protons with the deuterium ions from the solvent (15). The kinetics for the deuterium exchange of I were studied using PMR spectroscopy, and conditions were developed for the selective preparation of II and III using trifluoroacetic acid-*d*. The ester and silyl ether derivatives of I were used to study the electron-impact and chemical-ionization mass spectral characteristics of II and III for potential use as internal standards in qualitative and quantitative determinations of I. Finally, II was employed to analyze the ^{13}C-NMR spectrum of I.



I: R = R′ = H
II: R = R′ = D
III: R = H, R′ = D

RESULTS AND DISCUSSION

The deuteration of apomorphine (I) in trifluoroacetic acid-*d* was studied at various temperatures and in the presence of a transition metal catalyst (5% rhodium on aluminum oxide). Products with increasing deuterium substitution at the aromatic positions were isolated (Table I). PMR spectroscopy was used primarily to determine the sites of substitution, while the extent of incorporation was assessed through mass spectral and PMR analysis. When I was dissolved in trifluoroacetic acid-*d*, there was a collapse of the pair of doublets at δ 6.72 and 6.92 in the PMR spectrum, which is attributable to the exchange of the C-8 and C-9 protons, respectively. Careful analysis of this exchange in excess trifluoroacetic acid-*d* showed that it followed first-order kinetics (Fig. 1) and that C-8 H exchanged significantly faster ($t_{1/2}$ 69.4 min) than C-9 H ($t_{1/2}$ 115 min). This later observation may reflect differences in the resonance stabilization of cationic transition-state intermediates at C-8 and C-9 and correlates with previous work on the bromination of apomorphine dimethyl ether (16).

The protonated amine of apomorphine hydrochloride also underwent exchange in trifluoroacetic acid-*d* as expected. However, it was surprising that the exchange of the amine proton was more than one order of magnitude slower (k = 0.0325 hr^{-1}, r = 0.996, a = 87%, $t_{1/2}$ = 21.3 hr) than the exchange of the aromatic protons at C-8 and C-9.

In contrast to the C-8 and C-9 protons, the exchange of the C-1 and C-3 protons for deuterium in trifluoroacetic acid-*d* was relatively slow at room temperature. With PMR spectroscopy, it appeared that no more than a 15% exchange occurred at C-1 and C-3 in excess trifluoroacetic acid-*d*, even after 216 hr. Elevated temperatures were required to prepare a product significantly enriched in II, and no improvement in incorporation was observed with a rhodium catalyst (Table I). The exchange of the C-1 and C-3 protons for deuterium was observed through loss of the doublets at δ 8.15 and 7.11, respectively. These changes were accompanied by an expected collapse of the triplet observed for C-2 H at δ 7.31 into a singlet integrating for one proton.

The noted assignments parallel those made by Ginos *et al.* (13) for I and II in dimethyl sulfoxide-d_6. These investigators studied the deuteration of apomorphine in 85% D_3PO_4 (in deuterium oxide) and found that II was formed after heating at 95° for several hours or at 140° for 30 min. Convincing PMR evidence was provided for the purity and assign-

Table I—Deuteration of Apomorphine

Deuterated Analog of I	Percent Formation under Various Conditions[a]				
	A	B	C	D	E
d_0	—	—	—	1	—
d_1	10	11	4	6	1
d_2	83	85	19	22	7
d_3	7	2	41	35	32
d_4	—	2	30	29	44
d_5	—	—	6	6	16
Atom percent of deuterium[b]	39	39	63	60	74
Percent of theoretical yield[c]	84	14	42	40	44

[a] Key: A, conditions as indicated under *Experimental* for III; B, same as A only with 50 mg of 5% rhodium on aluminum oxide; C, 5 ml of trifluoroacetic acid-*d* and 0.66 mmole of I·HCl with agitation at 140° for 72 hr; D, identical to C only with 50 mg of 5% rhodium on aluminum oxide; and E, conditions as indicated under *Experimental* for II. [b] Calculations are based on mass spectral analysis and are the atom percent of deuterium of aromatic protons only. [c] Isolated product.

0022-3549/80/0900-1040$01.00/0
© 1980, American Pharmaceutical Association

Table II—Mass Spectral Characteristics of Apomorphine, Its Deuterated Analogs, and Some Potentially Suitable Derivatives for GLC–Mass Spectral Analysis

Compound	Ionization Mode	Scanned	Base Peak	Parent or Pseudoparent Ion (Relative Abundance)	$[M-1]^+/M^+$	Other Important Fragments (Relative Abundance)
I (free base)	EI	36–350	266	267 (64)	1.55	—
I·HCl	EI	50–350	266	267 (72)	1.39	—
II·HCl	EI	70–310	270	271 (75)	1.33	—
III·HCl	EI	70–350	268	269 (70)	1.43	—
I·HCl	CI[a]	70–350	268	268 (100)	—	267 (9)
III·HCl	CI	70–350	270	270 (100)	—	269 (25)
I heptafluorobutyrate[b]	EI	100–730	169	659 (37)	1.03	658 (38)
I trimethylsilyl ether[c]	EI	100–450	410	411 (72)	1.38	322 (79)
II trimethylsilyl ether[c]	EI	100–450	414	415 (90)	1.11	326 (80)
III trimethylsilyl ether[c]	EI	100–450	412	413 (75)	1.33	324 (66)
I trimethylsilyl ether[c]	CI	100–450	412	412 (100)	—	396 (61)
II trimethylsilyl ether[c]	CI	100–450	416	416 (100)	—	400 (46)
III trimethylsilyl ether[c]	CI	100–450	414	414 (100)	—	398 (34)
I *tert*-butyldimethylsilyl ether[d]	EI	100–530	322	495 (4)	0.3	438 (15)

[a] Essentially identical results were obtained when methane or isobutane was used as the ionizing gas. All other chemical-ionization determinations were made with methane. [b] *O,O*-Bis(heptafluorobutyrate) ester. [c] *O,O*-Bis(trimethylsilyl) ether. [d] *O,O*-Bis(*tert*-butyldimethylsilyl) ether.

ment of deuterium atoms in II. However, mass spectral examination of this product proved to be somewhat anomalous; the mass spectrum indicated that ~55% of the aromatic protons had been exchanged for deuterium (d_0, 23; d_1, 0; d_2, 11; d_3, 19; d_4, 41; and d_5, 6), while the PMR spectrum indicated that 80% of the aromatic protons had been exchanged. The relatively high proportion of undeuterated product shown by the mass spectrum was attributed to the exchange between the proton from hydrogen chloride and the deuterium atoms on the aromatic rings (II was analyzed as its hydrochloride salt) during vaporization in the mass spectrometer. The PMR determinations of II and III performed during the present studies were in agreement (within ±4%) with the compositions estimated by mass spectral analysis (Table I).

The exchanges of the C-1 and C-3 protons in trifluoroacetic acid-*d* were facile compared to the exchange of C-2 H. Indeed, no exchange of C-2 H could be detected when I was treated with trifluoroacetic acid-*d* (Table I) or allowed to stand in trifluoroacetic acid-*d* for 9 days at 25°. The ease of exchange of C-1 and C-3 H relative to C-2 H probably results from the influence of the 11-hydroxyl group, which causes resonance stabilization of the developing positive charges at C-1 and C-3. In contrast, a resonance interaction of the 10-oxygen atom across both rings is prevented because of its *meta*-relationship to the A-ring. Furthermore, the 10-hydroxyl group of I may be protonated preferentially in trifluoroacetic acid, which could have important implications for selective *O*-acylations of I in this solvent (17).

Since II and III are proposed for use in biological disposition studies, indication of their stability (with respect to the deuterium–hydrogen exchange) in aqueous media was desired. To determine if the aromatic protons of I at C-8 and C-9 would exchange under mildly acidic conditions, ~60 μmoles of I·HCl was dissolved in 1 ml of 0.02 and 0.3 *M* ascorbic acid (to prevent oxidation of I) prepared in deuterium oxide (pH 2.92 and 2.28, respectively) and stored in screw-capped test tubes at room temperature for 14 days. No incorporation of deuterium was detected after this treatment following GLC–mass spectral analysis of the isolated product as its bis(trimethylsilyl) ether derivative.

The use of II and III as GLC–mass spectral internal standards in metabolic studies requires derivatization to improve volatility and prevent thermal decomposition. Furthermore, the production of suitable (high molecular weight) fragment or parent ions that retain the deuterium label is desired, and these ions should be formed in sufficient amounts (*i.e.*, they should represent a significant proportion of the total ionization current) to permit sensitive detection. Previous work with I (18, 19) indicated that the *O,O*-bis(trimethylsilyl) ether and the *O,O*-bis(heptafluorobutyrate) ester derivatives are readily formed quantitatively and may be applicable to GLC–mass spectral determinations. Compounds converted to *tert*-butyldimethylsilyl ethers frequently form intense ions at high *m/e* values due to loss of a *tert*-butyl radical (M − 57) using electron-impact (EI) conditions, and this derivative was evaluated as well. The electron-impact and chemical-ionization (CI) mass spectral characteristics of I–III and their derivatives are presented in Table II.

A common fragment ion observed in the electron-impact mass spectrum of aporphines is an $[M-1]^+$ species, which is due to loss of the 6a-proton. As indicated in Table II, I and its derivatives, except for apomorphine *O,O*-bis(*tert*-butyldimethylsilyl) ether, give rise to a

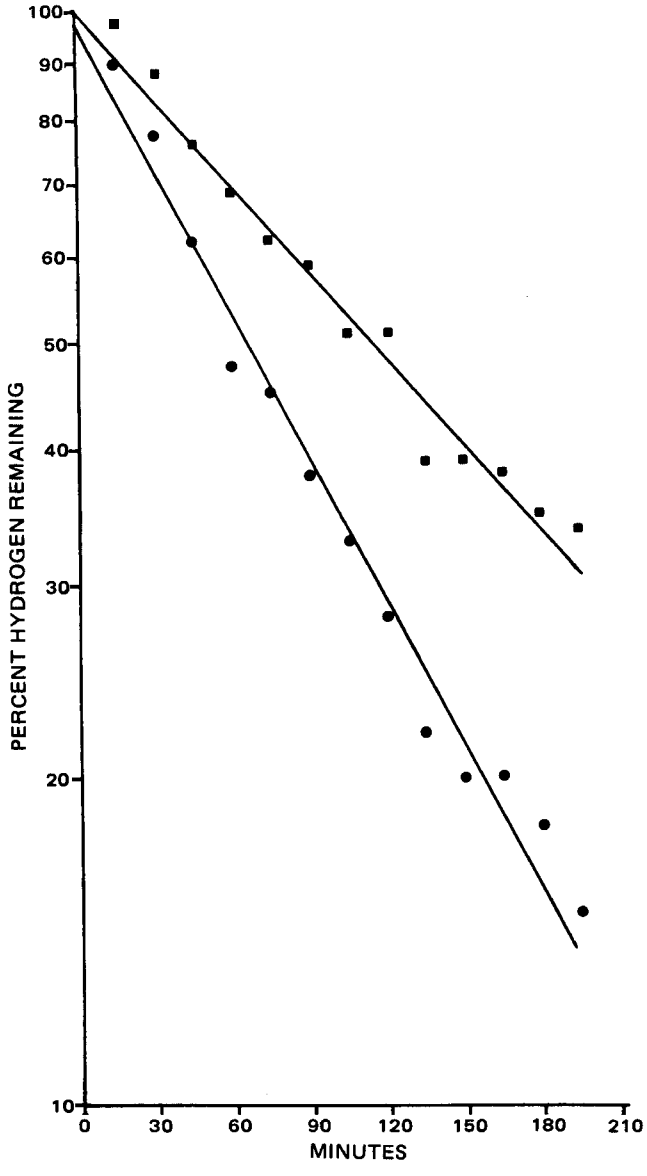


Figure 1—*Kinetics of exchange of C-8 H* (●) (k = *0.60 hr*$^{-1}$, r = *0.992*, a = *97.0%*, $t_{1/2}$ = *69.4 min*) *and C-9 H* (■) (k = *0.36 hr*$^{-1}$, r = *0.989*, a = *101.0%*, $t_{1/2}$ = *114.6 min*) *of apomorphine in trifluoroacetic acid-d at 25°.*

Table III—^{13}C-NMR Spectral Characteristics of Apomorphine in Trifluoroacetic Acid

Carbon	Chemical Shift, δ	Integrated Area[a]
C-1	130.8	2529
C-1a	130.8	—
C-1b	128.9	1265
C-2	129.2	—[b,c]
C-3	129.7	—[c,d]
C-3a	133.5	912
C-4	27.5	860
C-5	55.4	840
C-6a	65.7	1004
C-7	33.4	731
C-7a	127.5	1049
C-8	122.4	—[c,e]
C-9	118.0	940
C-10	143.7	957
C-11	144.8	1031
C-11a	122.1	—[f,g]
N-Methyl	43.5	1183

[a] From broad-band decoupled spectrum. [b] Not sufficiently resolved from C-1b for accurate area measurement. [c] Peak height significantly less than that of quaternary carbons. [d] Not sufficiently resolved from C-2 for accurate area measurement. [e] Not sufficiently resolved from C-11a for accurate area measurement. [f] Not sufficiently resolved from C-8 for accurate area measurement. [g] Peak height significantly greater than aromatic carbons bearing hydrogens.

prominent $[M - 1]^+$ peak. The bis(heptafluorobutyrate) derivative of I under electron-impact conditions gave a moderate parent ion (*m/e* 659) in addition to extensive formation of the imine fragment (*m/e* 658). However, a drawback in using the heptafluorobutyrate derivative is the possibility of exchange of the deuterium atoms at C-8 and C-9 due to small quantities of heptafluorobutyric acid present during derivatization (19).

The electron-impact mass spectrum of the *O,O*-bis(trimethylsilyl) ether of I showed a parent ion that was 75% of the base peak and accounted for 3% of the total ion current. A strong fragment ion was observed at *m/e* 322 for apomorphine as its trimethylsilyl derivative, and corresponding ions at *m/e* 326 and 324 were noted for the same derivative of II and III, respectively. However, in all of these cases, the fragment-ion peaks represented <10% of the total ion currents.

When I was treated with excess *tert*-butyldimethylimidazole, it formed a diether derivative, as evidenced by a parent peak at *m/e* 495. The formation of this derivative was somewhat unexpected since there is significant steric crowding in the resulting derivatized catechol. As anticipated, the parent ion was rather weak in the electron-impact mass spectrum of I as its *tert*-butyldimethylsilyl ether derivative, but there was a fragment ion of moderate intensity at *m/e* 438 ($[M - 57]^+$), which probably arose due to the loss of a *tert*-butyl radical (20). This fragment ion was 15% of the base peak but accounted for only 1% of the total ionization current. A second fragment ion at *m/e* 322 served as the base peak but represented only 6% of the total ionization current. The intensity of these ions may have been enhanced by the use of lower ionization potentials; however, the use of the *tert*-butyldimethylsilyl derivative in GLC–mass spectral work is complicated by its GLC characteristics. Even on a short column (50 cm, 3% methylphenylsiloxane; see *Experimental*), this derivative has long retention times (*e.g.*, 44 min at 210° with a carrier flow rate of 20 ml/min).

Since none of the derivatives had the desired characteristics for quantitative GLC–mass spectral work employing electron-impact conditions, chemical-ionization mass spectrometry was evaluated. The chemical-ionization mode has two advantages over electron-impact spectra. First, it usually is more sensitive than the electron-impact mode. In a typical electron-impact spectrum, the base peak may account for only 20% or less of the total ion current, while many chemical-ionization spectra display base peaks representing as much as 70–80% of the total ion current. Apomorphine proved to be no exception. With the electron-impact mode, the base peak for I was at *m/e* 266 ($[M - 1]^+$) and was 22% of the total ion current; with chemical-ionization mass spectrometry (isobutane), the base peak at *m/e* 268 ($[M + 1]^+$) was 59% of the total ion current.

A second advantage to the chemical-ionization mode is that it often gives a strong pseudoparent ion ($[M + 1]^+$), which may, because of its high molecular weight, be less likely to suffer interference from other components of biological extracts. Apomorphine and its dideuterated analog, III, gave intense pseudoparent ions at *m/e* 268 and 270, respectively, which were the base peaks in both cases. Even when isobutane was used, M^+ and $[M - 1]^+$ ions at *m/e* 266 and 267, respectively, were observed in the chemical-ionization mass spectrum of I. These ions were in approximately the same relative proportion to one another as seen in the electron-impact mass spectrum of I. It has been observed, especially with tertiary amines using methane as the reactant gas, that the amine can undergo electron transfer with one of the reactant ions, giving an M^+ ion (21, 22). This transfer apparently occurs with I. A similar observation was made with glaucine, a related aporphine (23). This observation restricts the use of underivatized I for qualitative work because the noted phenomenon could lead to errors in quantitative determinations.

Of the three derivatives studied, the trimethylsilyl ethers were the most suitable for GLC–mass spectral work. With methane as the GLC carrier gas and the chemical-ionization reactant gas, the chemical-ionization mass spectrum of the trimethylsilyl ethers of I, II, and III showed base peaks at *m/e* 412, 416, and 414, respectively; the ions accounted for 25–32% of the total ion currents for these compounds. However, as was seen with underivatized I, the trimethylsilyl ethers underwent electron transfer with the reactant ions, giving appropriate M^+ and $[M - 1]^+$ ions (*m/e* 411 and 410 for I as its trimethylsilyl derivative) that were quite intense (59 and 29% of the base peak, respectively, for the trimethylsilyl derivative of I). In addition, there appeared to be significant hydrogen abstraction, most likely of the 6a-proton. This phenomenon is observed with tertiary amines in which the hydride loss is enhanced by referring the charge to an adjacent nitrogen atom (22, 24), and it makes the pseudoparent ions of the trimethylsilyl ethers of I–III less desirable for selective-ion monitoring. Fortunately, there are strong and clean fragment ions for the aporphines as their trimethylsilyl derivatives at *m/e* 396 (I), 400 (II), and 398 (III) which account for ~15% of the total ion current in each case. These ion fragments probably occur through the loss of methane from pseudoparent ions, probably from the trimethylsilyl groups. In conclusion, it appears that II and III can function as internal standards for I if chemical-ionization mass spectrometry is used and the noted fragment ions of their trimethylsilyl derivatives are monitored.

The availability of II presented an opportunity to elaborate chemical shifts of the proton-bearing carbons of I. By use of broad-band decoupling, off-resonance white noise decoupling, and selective-proton decoupling, the ^{13}C-NMR data listed in Table III were compiled.

The broad-band decoupling spectrum of I contained only 16 lines; however, the signal at δ 130.8 integrated for two carbons. The off-resonance white noise decoupling spectrum indicated that the signals at δ 144.8, 143.7, 133.5, 130.8, 128.9, 127.5, and 122.1 were nonprotonated carbons. Chemical shifts assigned to aliphatic carbons in I (Table III) were deduced from data reported previously for similar aporphines (25, 26). The ^{13}C-NMR spectrum of II had a single aryl CH signal at δ 129.2.

The use of selective-proton decoupling of the aromatic protons in trifluoroacetic acid presented some problems. Irradiation (0.3 w) at δ H 7.31 to enhance C-2 and δ H 7.11 to enhance C-3 caused enhancement of all of the protonated aromatic carbon signals. In contrast, irradiation at δ H 8.15 to enhance C-1 caused collapse of all of the protonated aromatic carbon signals represented by lines integrating for one carbon; this effect was especially noticeable for the signal at δ 129.7. However, during this experiment, the signal at δ 130.8 (which integrates for two carbons) still integrated for two carbons, indicating that the protonated carbon resonating at δ 130.8 is C-1. By default, the carbon signal at δ 129.7 corresponds to C-3.

Assignment of the carbon signals for C-8–C-11 are based on reported assignments in similar aporphines (25, 26). The carbon assignments for nuciferine and isocorydine suggest that C-11a (*ortho* to phenol) should be the farthest upfield while C-3a should be the farthest downfield of the signals possible for these nuclei. The remaining nonprotonated carbons are provided with proposed assignments (Table III) based on the chemical shifts suggested for analogous carbons in isocorydine (25).

An unusual observation was made with respect to carbon resonance intensities when trifluoroacetic acid was used as the solvent. All of the nonprotonated aromatic carbons possessed intensities greater than or equal to those of the protonated aromatic carbons (during broad-band decoupling); the opposite usually is true because of nuclear Overhauser enhancement. This observation may have general applicability in differentiating nonprotonated from protonated carbons and is under further investigation in these laboratories.

EXPERIMENTAL

Reagents—(*R*)-(−)-Apomorphine hydrochloride hemihydrate was used as purchased[1]. Trifluoroacetic acid-*d* was generated from trifluo-

[1] McFarland Smith Ltd., Edinburgh, Scotland.

roacetic anhydride[2] with deuterium oxide[3] (99.7 atom % D). All other solvents and reagents were analytical reagent grade.

Mass Spectral and GLC–Mass Spectral Studies—Mass spectra were determined in the electron-impact and chemical-ionization modes using an automated GLC–mass spectrometric[4] system consisting of a microprocessor-controlled gas chromatograph coupled (all glass jet separator) to a quadrapole mass spectrometer and data system. Mass spectra of I–III (free base and hydrochloride salts) were obtained by introduction of the sample on a solid probe programmed from 100 to 300° (30°/min). GLC–mass spectral determinations were implemented with a U-shaped glass column (50 cm × 2 mm i.d.) packed with 3% methyl phenyl siloxane on a specially treated silaceous support[5] (100–120 mesh).

Helium (20 ml/min) was the carrier gas in all electron-impact studies; methane was the carrier gas (20 ml/min) in most chemical-ionization work. GLC–mass spectral analyses were carried out with a column temperature of 180–220°. The mass spectrometer was operated with an electron energy of 70 ev, an emission current of 350 μamp, an ion source temperature of 220°, and a scan rate of 30 scans/min from *m/e* 50 or 100 to 700. The final spectrum of each compound was an averaged sum of 10 or more spectra with the average background subtracted.

Trimethylsilyl derivatives were prepared by dissolving ~1 mg of I, II, or III in 100 μl of *N,O*-bis(trimethylsilyl)acetamide and heating at 65° for 30 min. The *tert*-butyldimethylsilyl derivative of I was prepared by reaction of ~1 mg of I·HCl with 200 μl of *tert*-butyldimethylsilylimidazole reagent[6] at 65° for 1 hr. Apomorphine *O,O*-bis(heptafluorobutyrate) was formed by a method reported previously (18).

NMR Studies—PMR and kinetic studies were performed with a 100-MHz[7] instrument, and chemical shifts are reported relative to tetramethylsilane. The rates of deuterium exchange were determined through analyses of integrated areas for C-8 and C-9 H relative to C-2 H in apomorphine hydrochloride. When I·HCl was reacted for 9 days at 25° in trifluoroacetic acid-*d* (containing 2 mg of dithionite/ml to prevent oxidation of I), the integrated area for C-2 H relative to the aliphatic protons remained constant within experimental error.

^{13}C-NMR spectra were obtained with a 90-MHz Fourier transform system[8], and chemical shifts are reported relative to tetramethylsilane. Solutions containing 200 mg of I·HCl/ml of trifluoroacetic acid and 75 mg of II·HCl/ml of trifluoroacetic acid-*d* were analyzed with an external deuterium oxide capillary for locking. For selective-proton decoupling experiments, approximate proton chemical shifts relative to carbon resonances were determined from analysis of acetone-d_6 solutions of I·HCl. The sample for ^{13}C-NMR analysis then was irradiated at the appropriate chemical shift with the source set at 0.3 w.

[1,3,8,9-$^2H_4$]Apomorphine (II)—(*R*)-(−)-Apomorphine hydrochloride hemihydrate (400 mg, 1.2 mmoles) was dissolved in trifluoroacetic acid-*d* (5 ml, 67.3 mmoles) in a silylated 16 × 100-mm polytef-lined screw-capped culture tube and placed in a pipe bomb at 140° for 96 hr. The trifluoroacetic acid-*d* was removed under a nitrogen stream, leaving a pale-brown oil. The oil was treated with a saturated solution of sodium bicarbonate, and the mixture was extracted with ether (4 × 5 ml). The ether was removed under reduced pressure, and the residue was redissolved in dry ether (15 ml). The hydrochloride salt, obtained by bubbling hydrogen chloride through the ether solution, was filtered and dried under vacuum to give 88 mg (44% of theoretical yield) of pale-green powder. Recrystallization from methanol–acetone gave off-white to pale-green crystals.

Purity was >99% as determined by UV spectrophotometry using spectral comparisons and absorbance values at 273 nm from authentic I·HCl [λ_{max} (water) 273 nm, ϵ = 17,300]. The GLC retention time of II as its heptafluorobutyrate derivative was identical to that obtained from similarly derivatized I. The parent peak of II as the free base was at *m/e* 271 ($C_{17}H_{13}D_4NO_2$) with a base peak at *m/e* 270 ($C_{17}H_{12}D_4NO_2$) and a retro-Diels–Alder peak at *m/e* 228 ($C_{15}H_8D_4O_2$). PMR analysis (trifluoroacetic acid-*d*) showed a signal at δ 7.42 (s, C-2 H) and residual signals at δ 8.24 and 7.20 from I-d_3 (Table I).

[8,9-$^2H_2$]Apomorphine (III)—(*R*)-(−)-Apomorphine hydrochloride hemihydrate (200 mg, 0.66 mmole) was dissolved in trifluoroacetic acid-*d* (5 ml, 67.3 mmoles) and kept in the dark at room temperature for 72 hr. The workup was the same as that described for II and gave 140 mg (70% of theoretical yield) of III·HCl as an off-white to pale-green powder. Purity was >99% as determined by UV spectrophotometry, and the GLC retention time of the heptafluorobutyrate derivative of III was identical to that of the corresponding derivative of I. The parent peak of III as the free base was at *m/e* 269 ($C_{17}H_{15}D_2NO_2$), the base peak was at *m/e* 268 ($C_{17}H_{14}D_2NO_2$), and the retro-Diels–Alder peak was at *m/e* 226 ($C_{15}H_{10}D_2O_2$); PMR (trifluoroacetic acid-*d*): δ 8.24 (d, 1H, *J* = 8.0, C-1 H), 7.42 (t, 1H, *J* = 8.0, C-2 H), 7.20 (d, 1H, *J* = 8.0, C-3 H), 6.96 (s, C-9 H, due to the presence of a trace amount of C-8 $^2H_1$), and 6.83 (s, C-8 H, due to the presence of a trace amount of C-9 $^2H_1$) (Table I).

Use of Rhodium Catalyst—Reactions were performed as indicated except that 50 mg of 5% rhodium on aluminum oxide[9] was added and the reaction mixtures were agitated for the times indicated. Workup procedures were identical to those outlined.

REFERENCES

(1) R. S. Schwab, L. V. Amador, and J. Y. Littvin, *Trans. Am. Neurol. Assoc.*, **76,** 521 (1951).

(2) G. C. Cotzias, P. S. Papavasiliou, C. Fehling, B. Kaufman, and I. Mena, *N. Engl. J. Med.*, **282,** 31 (1970).

(3) G. C. Cotzias, P. S. Papavasiliou, E. S. Tolosa, J. S. Mendez, and M. Bell-Midura, *ibid.*, **294,** 567 (1976).

(4) C. D. Marsden, D. Tarsy, and R. J. Baldessarini, in "Psychiatric Aspects of Neurological Disease," D. F. Benson and D. Blumir, Eds., Grune and Stratton, New York, N.Y., 1975, pp. 219–265.

(5) E. S. Tolosa, *J. Am. Med. Assoc.*, **229,** 1579 (1974).

(6) E. S. Tolosa and S. B. Sparber, *Life Sci.*, **15,** 1371 (1974).

(7) C. A. Tamminga, M. H. Schaffer, R. C. Smith, and J. M. Davis, *Science*, **200,** 567 (1978).

(8) J. G. Cannon, R. V. Smith, A. Modiri, S. P. Sood, R. J. Borgman, M. A. Aleem, and J. P. Long, *J. Med. Chem.*, **15,** 273 (1972).

(9) R. V. Smith and A. W. Stocklinski, *Anal. Chem.*, **47,** 1321 (1975).

(10) R. V. Smith, P. W. Erhardt, J. L. Neumeyer, and R. J. Borgman, *Biochem. Pharmacol.*, **25,** 2106 (1976).

(11) W. H. Riffee, R. E. Wilcox, W. H. Soine, and R. V. Smith, *Pharmacologist*, **20,** 184 (1978).

(12) "Mass Spectrometry in Drug Metabolism," A. Frigerio and E. L. Ghisalberti, Eds., Plenum, New York, N.Y., 1977.

(13) J. Z. Ginos, A. LoMonte, G. C. Cotzias, A. K. Bose, and R. J. Brambilla, *J. Am. Chem. Soc.*, **95,** 2991 (1973).

(14) R. Gottlieb and J. L. Neumeyer, *ibid.*, **98,** 7108 (1976).

(15) W. H. Soine, P. Salgo, and R. V. Smith, *J. Labelled Compd. Radiopharm.*, **16,** 597 (1979).

(16) R. V. Smith and A. W. Stocklinski, *Tetrahedron Lett.*, **1973,** 1819.

(17) R. J. Borgman, R. V. Smith, and J. E. Keiser, *Synthesis*, **1975,** 249.

(18) R. V. Smith and A. W. Stocklinski, *Anal. Chem.*, **47,** 1321 (1975).

(19) D. M. Baaske, J. E. Keiser, and R. V. Smith, *J. Chromatogr.*, **140,** 57 (1977).

(20) R. W. Kelly and P. L. Taylor, *Anal. Chem.*, **48,** 465 (1976).

(21) M. S. B. Munson and F. H. Field, *J. Am. Chem. Soc.*, **88,** 2621 (1966).

(22) F. H. Field, in "Advances in Mass Spectrometry," vol. 5, A. Macoll, Ed., MTP International Review of Science, series 1, Butterworths, London, England, 1972, p. 133.

(23) H. M. Fales, H. A. Lloyd, and G. W. A. Milne, *J. Am. Chem. Soc.*, **92,** 1590 (1970).

(24) T. A. Whitney, L. P. Klemann, and H. F. Field, *Anal. Chem.*, **43,** 1048 (1971).

(25) E. Wenkert, B. L. Buckwalter, I. R. Burfitt, M. J. Gassie, H. E. Gottlieb, E. W. Hagaman, F. M. Schell, and P. M. Wovkulich, in "Topics in C-13 N.M.R. Spectroscopy," vol. 2, G. C. Levy, Ed., Wiley–Interscience, New York, N.Y., 1976, p. 105.

(26) M. Shamma, in "The Alkaloids—Specialist Periodical Reports," vol. 7, M. F. Grundon, Ed., The Chemical Society, London, England, 1977, pp. 152–166.

[2] Matheson, Coleman and Bell, Norwood, Ohio.

[3] Merck & Co., Rahway, N.J.

[4] Finnigan 4023 system complete with model 9610 gas chromatograph, model 4000 quadrapole mass spectrometer, and model 2300 INCOS data system, Finnigan Instruments, Sunnyvale, Calif.

[5] OV-17 on Chromosorb WHP, Analabs, North Haven, Conn.

[6] Applied Science Laboratories, State College, Pa.

[7] Model HA-100, Varian Associates, Palo Alto, Calif.

[8] Model WH 90, Bruker Instruments, Billerica, Mass.

[9] Pfaltz & Bauer, Stamford, Conn.

ACKNOWLEDGMENTS

Supported in part by Grant NS-12259 from the National Institute of Neurological and Communicative Disorders and Stroke and Grant GP-41570 from the National Science Foundation.

The authors are grateful to Peter Salgo for technical assistance.

Antitumor Agents XXXVI: Structural Elucidation of Sesquiterpene Lactones Microhelenins-A, B, and C, Microlenin Acetate, and Plenolin from *Helenium microcephalum*

YASUHIRO IMAKURA *, KUO-HSIUNG LEE *x, DONALD SIMS *, RONG-YANG WU *, IRIS H. HALL *, HIROSHI FURUKAWA ‡, MASATAKA ITOIGAWA ‡, and KAZUE YONAHA ‡

Received December 26, 1978, from the **Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514*, and the ‡*Faculty of Pharmacy, Meijo University, Tempaku, Nagoya, Japan.* Accepted for publication April 6, 1979.

Abstract □ The antitumor sesquiterpene lactones microhelenins-A, B, and C, microlenin acetate, and plenolin were isolated from *Helenium microcephalum*. The structures and stereochemistry of these lactones were determined by physical methods as well as by chemical transformations and correlations. Microlenin acetate appears to be the first novel dimeric sesquiterpene lactone demonstrated to have significant antileukemic activity.

Keyphrases □ Antitumor agents—microhelenins, microlenin acetate, and plenolin, isolation from *Helenium microcephalum*, determination of structure and stereochemistry □ Microhelenins—isolation from *Helenium microcephalum*, determination of structure and stereochemistry □ Sesquiterpene lactones—microhelenins, microlenin acetate, and plenolin, isolation from *Helenium microcephalum*, determination of structure and stereochemistry

Search for an ample supply of helenalin for investigation into the relationship between the structure of sesquiterpene lactones and their cytotoxic antitumor activity led to the use of the plant *Helenium microcephalum*[1] (1). Preexamination of the whole plant extract revealed that the removal of helenalin left a mother liquor, which retained significant inhibitory activity against Walker 256 carcinosarcoma in rats and P-388 lymphocytic leukemia in mice. Further work with the chloroform extract resulted in the isolation[2] and structural determination of the new antitumor agents microhelenins-A (I) (2), B (VI) (3), C (VIII) (3), and D (mexicanin-E, XIV) (3) and microlenin[3] (4, 5), which were described in preliminary reports.

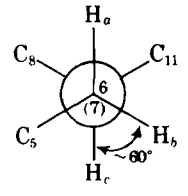
The purpose of this paper is to describe fully the isolation and structural elucidation of microhelenins-A, B, and C, the new dimeric antileukemic agent microlenin acetate[3] (X), and the companion pseudoguaianolide plenolin (VII) (7).

RESULTS AND DISCUSSION

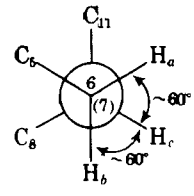
The final chloroform extract of the whole plant of *H. microcephalum* was chromatographed on silica gel using chloroform and chloroform–ethyl acetate (9:1) as the eluents. The initial chloroform eluate afforded a gum of several components. Further silica gel column chromatography and preparative TLC of this mixture led to the isolation of microhelenins-A, B, C, and D. Subsequent elution with chloroform–ethyl acetate (9:1) gave plenolin and microlenin as well as helenalin. Microlenin acetate was isolated from the mother liquor after the removal of helenalin.

Microhelenin-A (I)—Microhelenin-A (I), $C_{15}H_{18}O_4$ (high-resolution mass spectral and elemental analyses), mp 140–141°, $[\alpha]_D^{24}$ +89.0°, showed the partial structure A, with the lactone ring closed at C-7 and C-8 and the methylene group of CH_2OCH attached at C-5 on the basis of IR and NMR (Table I) evidence as reported previously (2).

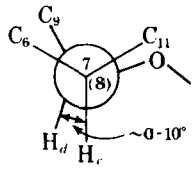
The circular dichroism and optical rotatory dispersion data of microhelenin-A are listed in Table II together with data for 2,3-dihydrohelenalin (II) (8), whose absolute configuration has been established. Similarities in the sign and magnitude of ketone Cotton effects indicated that I possessed the same *trans*-fused cyclopentanone ring system (C-1α, C-5β-CH_2O) as II. Determination of the *cis*-stereochemistry of the γ-lactone ring in I was based on the generalization that *cis*-fused lactones closed toward C-8, exhibiting a positive lactone Cotton effect, as exemplified by II (9, 10). In addition, Samek's rule (11) ($J_{7,13}$ *trans*-lactone $\geq 3 \geq J_{7,13}$ *cis*-lactone), which generally has been applicable to guaianolides, also indicated that I contains a *cis*-fused lactone since $J_{7,13e}$ = 2.5–3.0 and $J_{7,13f}$ = 2.0–3.0[4]. This evidence led to postulation of possible conformations of the seven-membered ring of I (C and D).



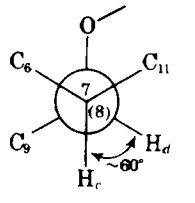
C: boat and half-chair



D: chair



E: boat



F: half-chair

[1] The constituents of *H. microcephalum* were examined previously and reported to contain helenalin in good yield (1).

[2] Helenalin also was isolated from this chloroform extract.

[3] Microhelenins-A, B, C, and D and microlenin showed significant (T/C ≥ 125%) inhibitory activity against Walker 256 carcinosarcoma in rats at T/C = 148, 138, 159, 144, and 172% at 2.5 mg/kg, respectively. Microlenin and microlenin acetate demonstrated significant (T/C ≥ 120%) inhibitory activity against P-388 lymphocytic leukemia in mice at T/C = 167 and 147% at 12.5 mg/kg, respectively, according to a literature method (6). Plenolin showed T/C = 138% at 25 mg/kg in the P-388 screen.

[4] The coupling constants of $J_{7,13}$ were changed by the conditions of the NMR instrument (XL-100), as shown in the data.

0022-3549/80/0900-1044$01.00/0
© 1980, American Pharmaceutical Association