

STUDIES ON THE ULEINE ALKALOIDS I. CARBON-13 NMR STUDIES ON ULEINE, 20-EPIULEINE AND (4S)-ULEINE-N_b-OXIDE¹

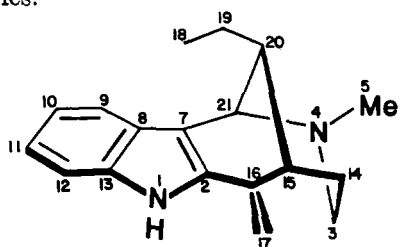
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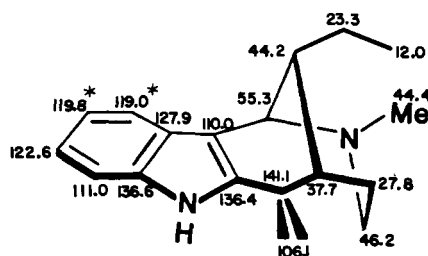
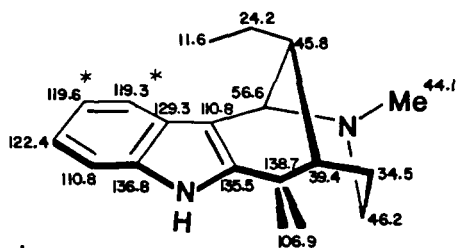
ABSTRACT.—An internally consistent set of carbon-13 assignments for uleine (1) and two closely related derivatives, 20-epiuleine (2) and (4S)-uleine-N_b-oxide (3), are presented.

Uleine (1) was first obtained from a methanol extract of the root bark of *Aspidosperma ulei* Mgf. (1), and the structure (2) and stereochemistry (3,4) were deduced subsequently. Confirmation of the structure was made through stereo-controlled synthesis (5,6).

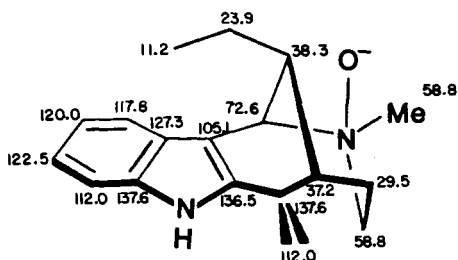
In the course of studies directed toward a biomimetic synthesis of the antitumor alkaloid ellipticine, it became necessary to unambiguously assign the carbon-13 nmr spectrum of the indole alkaloid uleine (1). We report here complete assignments for the three alkaloids uleine (1), 20-epiuleine (2), and uleine-N_b-oxide (3). No carbon-13 nmr data have previously been reported for compounds in this series.



1 Uleine



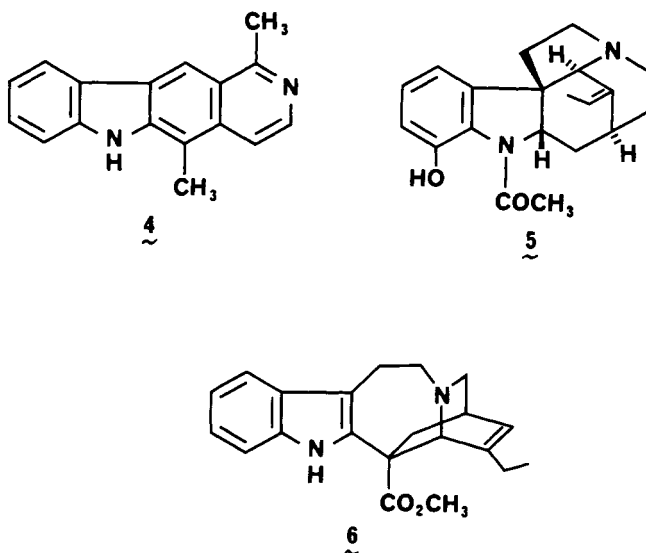
2 20-Epiuleine



3 4S-Uleine-N_b-oxide

For the purposes of this study, uleine (1) and 20-epiuleine (2) were isolated from an extract of the bark of *Aspidosperma subincanum* K. von Mart. (Apocynaceae), and (4S)-uleine-N_b-oxide (3) was produced by peroxide oxidation of 1. None of the (4R)-isomer was produced during this oxidation as evidenced by both proton and carbon-13 nmr studies. During the course of the isolation, olivacine (4) and limatinine (5) were also isolated (7, 8).

¹Portions of this work were submitted by R.P.B. in partial fulfillment of the Ph.D. degree requirement of the Graduate College, University of Illinois at the Medical Center, August, 1981, and were first presented at the Joint Meeting of the American Society of Pharmacognosy and the Society for Economic Botany, Boston, Mass., July, 1981.



RESULTS AND DISCUSSION

The carbon-13 nmr spectra of uleine, 20-epiuleine and (4*S*)-uleine-*N*_b-oxide in the SFORD mode were each expected to contain two quartets, three triplets and three doublets in the region upfield of CDCl₃. The resonances and assignments for the three compounds from spectra determined in CDCl₃ at 22.68 MHz are given on the structures. A number of the assignments merit particular discussion.

In all three compounds, the assignment of resonances for C-7, C-8, C-9, C-10, C-11 and C-12 was made by analogy with values for similar carbon atoms in catharanthine (6) (9), although ambiguity remains for C-9 and C-10 in 1 and 2. Assignment of C-17 was trivial as it is the only sp² carbon bearing two protons and, therefore, the only triplet downfield of CDCl₃ in the SFORD or coupled ¹³C spectrum. The methyl groups, C-18 and C-5, could likewise readily be assigned by inspection in all cases. Carbon-18, like its protons, resonates at higher field in uleine than in 20-epiuleine, presumably due to a combination of steric and ring-current effects.

Assignment of the aliphatic methine and methylene resonances posed more demanding problems. The most downfield methylene in the aliphatic region could be assigned to C-3, the only methylene bearing nitrogen. Comparison of the frequencies of the remaining two methylenes in uleine and the derivatives permitted assignment of these resonances in each compound. In 20-epiuleine, the ethyl side chain is axial with respect to the piperidine ring, whereas in uleine, it is equatorial. Thus, a 1,3-diaxial (γ) interaction would be expected to occur between the ethyl group and the axial proton at C-14. This would result in a steric compression of C-14, C-20 and to a lesser extent C-18 and C-19 in 20-epiuleine and should lead these carbons to resonate at higher field in 20-epiuleine than in uleine (10,11). Examination of the carbon-13 data indicates that the carbon signal δ 24.2 is shifted upfield to δ 23.3. Thus the methylene at δ 34.5 in uleine, which shows a gamma effect due to steric compression of nearly 7 ppm, can be assigned to C-14; and the triplet at δ 24.2 in uleine, which shows a much smaller steric compression in the epimer, can be assigned to C-19.

In (4*S*)-uleine-*N*_b-oxide a similar situation exists, where the introduction of an axial oxygen would be expected to produce an upfield shift in the resonance of C-14 and C-20 due to steric and inductive effects (10,11) but little effect on C-19. Indeed, the resonance assigned to C-14 shifts upfield by 5.0 ppm on conversion

to the N₁-oxide; the resonance assigned to C-20 shifts 7.5 ppm; while the C-19 resonance shifts only 0.3 ppm.

Similar arguments were used in the assignment of the aliphatic methine carbon resonances. The most downfield doublet in the respective aliphatic regions was assigned to C-21, the only methine carbon bearing nitrogen. With N-methyl-piperidine and N-methyl-piperidine-N-oxide (9) as model systems, a substantial upfield shift in the resonance of C-20 would be expected on going from uleine to its N₁-oxide, while a much smaller shift would be expected for C-15. The observed data indicate that the resonance at δ 45.8 in uleine shifts to δ 38.3 in the N₁-oxide ($\Delta\delta$ -7.5), whereas the resonance at δ 39.4 in uleine is shifted only to δ 37.2 in the N₁-oxide ($\Delta\delta$ -2.2). Thus the latter signal may be assigned to C-15 and the former to C-20.

Observed data for 20-epiuleine are in agreement with these assignments. Thus the signal for C-20 (δ 44.2) is 1.6 ppm upfield of the corresponding signal in uleine, consistent with a steric compression effect resulting from the 1,3-diaxial (gamma) interaction between the ethyl side chain and the axial proton of C-14. Upfield shifts of the resonances of C-15 and C-21 (1.7 and 1.3 ppm, respectively) are probably due to an increase in strain in the piperidine ring resulting from this interaction. With these assignments in hand, attention was focused on the downfield quaternary carbons.

Additivity parameter calculations (10) suggest that the resonance for C-16 should be the most downfield signal in the carbon-13 nmr spectrum of each of these compounds, followed by C-13 and then C-2. Since use of these calculations in poly-functional compounds is not always reliable, independent information on these assignments was sought by comparison of the chemical shifts of these carbon atoms in uleine and 20-epiuleine, with the chemical shift of C-7 which can be readily assigned in each compound. Carbon-7 experiences a downfield shift of 2.43 ppm between uleine and 20-epiuleine, consistent with a release of strain in the cyclohexene ring and/or removal of steric compression. If it can be assumed that the release of ring strain would be felt equally at symmetrically displaced centers, a downfield shift of similar magnitude might reasonably be expected for C-16. Indeed, the signal tentatively assigned to C-16 in uleine (δ 138.7) shifts downfield by 2.4 ppm in 20-epiuleine, while the signal for C-2 (δ 135.5) shifts downfield by 0.9 ppm and that for C-13 remains virtually unchanged.

Confirmation of these assignments was derived from the coupled (gated decoupling) carbon-13 nmr spectrum of uleine in the presence and absence of deuterium oxide (fig. 1). In the absence of D₂O, the most downfield signal (δ 138.7) appears as a narrow multiplet, while the next two multiplets to higher field (δ 136.8, 135.5) are somewhat broadened. On the addition of D₂O, these multiplets sharpen appreciably, while the downfield signal remains unchanged. This difference establishes that the two higher field signals are directly bonded to nitrogen and that these carbons are coupled to the indole nitrogen proton. As a result of this exchange, the most downfield signal can be unequivocally assigned to C-16. In the presence of deuterium oxide, the multiplet at δ 136.8 collapses to a clean triplet ($^3J_{CH}$ = 7.6 Hz), indicating that this signal corresponds to C-13, which is expected to couple with H-9 and H-11. The more complex pattern observed in the multiplet at δ 135.16 is consistent with the coupling of C-2 to H-15, H-17 and H-21. The coupling $^3J_{CNH}$ was estimated to be 4.9 Hz for C-8 and 3.7 Hz for C-13. Tables 1 and 2 summarize the direct bond and long-range aromatic coupling constants observed for uleine (1).

Through the examination of broad-band decoupled, single-frequency off-resonance decoupled and gated decoupled spectra, unambiguous assignments of the carbon resonances in uleine (1), 20-epiuleine (2) and (4S)-uleine-N₁-oxide (3) were achieved. Deuterium exchange of the indole NH in the gated decoupled spectrum was critical in assigning certain of the quaternary aromatic carbon frequencies.

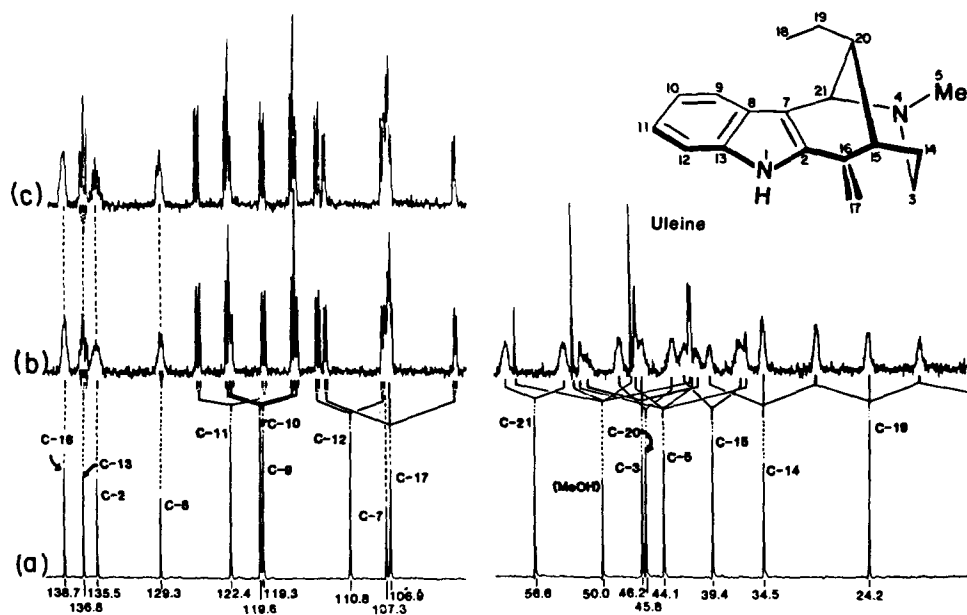


FIG. 1. (a) Broad-band decoupled spectrum of uleine (1)
 (b) Gated-decoupled spectrum of uleine (1)
 (c) Addition of D_2O to spectrum (b).
 Spectra were recorded at 25.05 MHz in $CDCl_3$ with TMS as an internal standard.

This is the first report of the analysis of the carbon-13 nmr spectra of any compound having the dasycarpidan skeleton.²

EXPERIMENTAL³

PLANT MATERIAL AND INITIAL FRACTIONATION.—An extract of *Aspidosperma subincanum* K. von Mart. (Apocynaceae) used in these studies was generously provided by Dr. J. A. Joule, University of Manchester.⁴ The ethanol extract (185 g) was dissolved in 2% aqueous tartaric

TABLE 1. Direct bond $^1J_{C,H}$ coupling constants for uleine.

Carbon No.	$^1J_{C,H}$ (Hz)	Carbon No.	$^1J_{C,H}$ (Hz)
C-3	133.4	C-15	135.1
C-5	133.4	C-18	124.6
C-9	159.7	C-19	126.4
C-10	159.7	C-20	129.9
C-11	159.7	C-21	140.4
C-12	158.0	(MeOH)	140.4
C-14	128.1		

²During the course of publication partial carbon-13 data on a dihydrouleine were published (E. C. Miranda and S. Blechert, *Tetrahedron Letts.*, **23**, 5395 (1982)).

³Melting points were determined on a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G spectrophotometer, and the ir spectra with a Beckman model IR 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1} ; absorption bands are reported in wave number (cm^{-1}). Proton NMR spectra were recorded in $CDCl_3$ with a Varian T-60A instrument operating at 60 MHz with a Nicolet Model TT-7 Fourier Transform attachment. Carbon magnetic resonance spectra were recorded in $CDCl_3$ with a Bruker WH-90 instrument operating at 22.68 MHz or a Jeol FX-100 spectrometer at 25.05 MHz. Carbon assignments were confirmed by analysis of the single-frequency off-resonance decoupled (SFORD) spectra or by analysis of the proton coupled ^{13}C spectra. Tetramethylsilane was used as an internal standard for both proton and carbon spectra and chemical shifts are recorded in δ (ppm). Mass spectra were obtained with a Varian MAT 112S double focusing spectrometer operating at 70 eV.

⁴The extract was prepared in September 1965 by Soxhlet extraction of bark material collected near Mariquita in the state of Bahia, Brazil ($44^{\circ}15'W$, $12^{\circ}25'S$) by Professor B. Gilbert. A voucher specimen is deposited in the herbarium of the Rio de Janeiro Botanical Garden.

TABLE 2. Summary of long-range ^{13}C - ^1H coupling constants for aromatic carbons of uleine.

Carbon No.	$(^2J_{\text{C,H}})$	$(^2J_{\text{C,NH}})^a$	$(^3J_{\text{C,H}})$
C-8	N.M. ^b	4.9	7.3
C-9	1.8		6.7
C-10	1.8		6.7
C-11	1.8		7.9
C-12	1.8		7.3
C-13		3.7	7.6

^aConfirmed by deuterium exchange.

^bNot able to be measured.

acid (2.4 liters) and extracted with water-saturated ethyl acetate (4 x 800 ml). After drying (Na_2SO_4), the pooled organic phases were evaporated to afford fraction A (21.24 g). Basification of the aqueous phase to pH 10 followed by extraction with water-saturated ethyl acetate (6 x 800 ml), drying (Na_2SO_4) the organic phase and evaporation afforded an alkaloidal fraction B (28.75 g). The aqueous phase was filtered, freed of organic solvent and lyophilized to afford a hygroscopic residue (fraction W, weight not determined).

ISOLATION OF ULEINE (1).—Fraction B was dissolved in warm methanol and allowed to stand overnight at 0° . The crystalline precipitate was filtered, washed with cold methanol and recrystallized from warm methanol to afford uleine (1, 6.64 g). An additional crop (1.52 g) was obtained through concentration of the mother liquor and methanol washings. The mother liquor was evaporated to dryness and held as fraction B, (18.92 g) for column chromatography. Uleine (1) exhibited the following physical and spectral properties: mp⁵ 74–98°, $[\alpha]^{24\text{D}} +17.1^\circ$ (CHCl_3); ir, ν_{max} (KBr), 3180, 2965, 2940, 1622, 1603, 1454, 1440, 1310, 997 and 725 cm^{-1} ; uv, λ_{max} (MeOH) 209 ($\log \epsilon$ 4.35), 310 (4.28), and 319 nm (4.26); ^1H -nmr, (CDCl_3 , 60 MHz) δ 0.84 (5H, m, 18-H₃, 19-H₂), 2.29 (3H, s, 5-H₃), 4.11 (1H, d, $J=2.3\text{ Hz}$, 21-H), 4.98 (1H, s, 17-H), 5.28 (1H, s, 17-H), 7.00–7.65 (4H, m, 4x Ar-H), and 8.60 (1H, bs, N-H); ms, m/z 266 (M^+ , 100%), 237 (64), 223 (41), 222 (23), 210 (26), 209 (95), 208 (51), 195 (42), 194 (85), 193 (32), 181 (35), and 180 (69).

ISOLATION OF OLIVACINE (4).—Fraction B, (18.92 g) was chromatographed over a column of Silica gel-60⁶ (500 g) packed in chloroform. Fraction 6, eluted with chloroform-methanol (97:3), afforded a yellow precipitate from chloroform identified as olivacine (167 mg), mp 315° (dec.); ^1H -nmr, (CD_3OD , 60 MHz), δ 2.81 (3H, s, Ar-CH₃), 3.08 (3H, s, Ar-CH₃), 7.16–7.50 (4H, m, 7-H, 8-H, 9-H, 11-H), 7.74 (1H, d, $J=6.6\text{ Hz}$, 3-H) and 8.73 (1H, br s, N-H); ms, m/z 246 (M^+ , 100%), 245 (36), 231 (5), 229 (5), 204 (8), and 123 (12). Identity was established by direct comparison with an authentic sample.

ISOLATION OF LIMATININE (5).—Fraction 9 from the above column, on preparative tlc on Silica gel-60 F₂₅₄ with chloroform-methanol (2:1) as the developing solvent, afforded an amorphous powder (67 mg) identified as limatinine (5): $[\alpha]^{24} +165^\circ$ (MeOH); ir, ν_{max} (KBr) 3200, 2935, 1622, 1598, 1568, 1470, 1433, 1245 and 779 cm^{-1} ; uv, λ_{max} (MeOH) 217 ($\log \epsilon$ 4.34), 258 (3.83), and 289 nm (3.45); ^1H -nmr (CDCl_3 , 60 MHz), δ 1.63 (3H, d, $J=6.8\text{ Hz}$, 18-H₃), 2.38 (3H, s, NCOCH_3), 4.34 (1H, dd, $J=6.9, 10.4\text{ Hz}$, 2-H), 4.50 (1H, s, 3-H), 5.45 (1H, q, $J=6.8\text{ Hz}$, 19-H), 6.63–7.22 (3H, m, 3 x Ar-H) and 10.23 (1H, br s, OH); ms, m/z 324 (M^+ , 24%), 281 (4.6), 137 (10), 136 (100) and 123 (8). Comparison of the observed spectral data with those published (7) for limatinine (5) confirmed the identity of the isolate.

ISOLATION OF 20-EPIULEINE (2).—Preparative tlc of the mother liquor and chloroform washings from above, on Silica gel-60 F₂₅₄ eluting with diethyl ether-methanol (3:1), afforded an amorphous gum (54.8 mg), identified as 20-epiuleine (2): $[\alpha]^{24\text{D}} +34.8^\circ$ (MeOH); ^1H -nmr (CDCl_3 , 60 MHz), δ 1.00 (3H, t, $J=6\text{ Hz}$, 18-H₃), 2.28 (3H, s, 5-H₃), 4.10 (1H, br s, 21-H), 4.96 (1H, s, 17-H), 5.24 (1H, s, 17-H), 7.00–7.70 (4H, m, 4 x Ar-H), and 8.62 (1H, br s, N-H); ms, m/z 266 (M^+ , 76%), 237 (54), 223 (31), 222 (17), 210 (22), 209 (69), 208 (37), 195 (32), 194 (69), 193 (24), 181 (36), and 180 (64). Additional quantities of 2 were obtained from adjacent fractions in a similar manner.

PREPARATION OF (4S)-ULEINE-N_b-OXIDE (3).—Hydrogen peroxide solution (30%, 5 ml) was added dropwise to uleine (1, 50 mg) in ethanol (95%, 10 ml) in an ice bath. After the mixture was stirred for 24 hrs, MnO_2 was added. The mixture, filtered through Celite and evaporated *in vacuo* at 40° , yielded a brown residue. Filtration of a chloroform solution of this material through neutral alumina⁷ (Brockman activity I) and removal of solvent afforded (4S)-uleine-N_b-oxide (3) as a yellow-brown amorphous gum (47.1 mg, 89%) possessing the following physical and spectral properties: $[\alpha]^{24\text{D}} +51.1^\circ$ (MeOH); ir, ν_{max} (KBr) 3100, 2960, 2940, 1642, 1613, 1500, 1450, 1322, 1058, and 910 cm^{-1} ; ^1H -nmr, (CDCl_3 , 60 MHz) δ 0.85 (5H, m, 18-H₃, 19-H₂),

⁵The wide range "melting point" of uleine reflects loss of the approximately one molecule of methanol per molecule of uleine which occludes during the crystallization process.

⁶E. Merck, Darmstadt, West Germany.

⁷M. Woelm, Eschwege, West Germany.

3.16 (3H, s, 5-H₃), 4.47 (1H, br d, $J=2$ Hz, 21-H), 5.22 (1H, s, 17-H), 6.15 (1H, s, 17-H), 7.00-7.60 (4H, m, 4 x Ar-H), and 12.37 (1H, br s, N-H); ms, m/z 232 (M^+ , 11%), 266 (27), 237 (17), 236 (10), 235 (11), 234 (12), 224 (18), 223 (100), 222 (54), 221 (12), 220 (11), 209 (35), 208 (57), 207 (29), 206 (25), 205 (11), 204 (15), 195 (17), 194 (39), 193 (16), 192 (11), 181 (14), 180 (29), and 167 (13).

No other isomers were detected through direct analysis of the reaction product. The mass spectrum of the product displayed a molecular ion at m/z 282, corresponding to the addition of the one oxygen atom to uleine. Proton nmr studies confirmed the identity of the product as an *N*-oxide of uleine, showing significant downfield shifts in the resonances for 21-H ($\Delta\delta +0.36$) and 5-H₃ ($\Delta\delta +0.87$). No doubling of these resonances was observed, supporting the contention that only a single isomer was present. Confirmation was subsequently obtained from the carbon-13 nmr spectrum discussed above.

Mechanistic considerations suggest that the configuration at position 4 (N_b) should be *S*, i.e., with oxygen introduced axially from the least hindered, β , face of the piperidine ring. The configuration of the *N*-oxide was established firmly as being *S* by comparison with the *R* isomer obtained as a metabolite of *Penicillium chrysogenum* (12).

ACKNOWLEDGMENTS

The authors would like to thank Dr. J. A. Joule, University of Manchester, Manchester, England, and Professor B. Gilbert, Instituto de Pesquisas da Marinha, Rio de Janeiro, Brazil, for an extract of *Aspidosperma subincanum* K. von Mart. and authentic samples and reference spectra of uleine, and Dr. C. R. Hutchinson, School of Pharmacy, University of Wisconsin, Madison, Wisconsin, for the preliminary 22.68 MHz carbon-13 nmr spectra of the isolates.

One of us (R.P.B.) would like to thank the American Foundation for Pharmaceutical Education for a graduate fellowship and the Graduate College, University of Illinois at the Medical Center for the award of a Dorothea H. Fleming Student Research Fellowship.

Received 7 June 1982

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