Synthesis, Characterization, and Analytical Studies of Adosupine, a Potential New Drug for Urinary Incontinence

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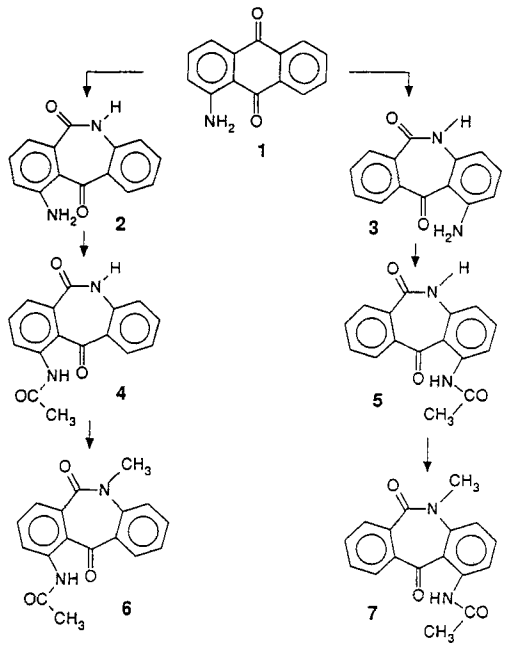
Abstract □ The synthesis and the spectroscopic characterization of a new potential drug for urinary incontinence, adosupine, is described. Adosupine and its potential synthesis impurities were analyzed by a new HPLC method that was developed with a C_{18} reversed-phase column. The analysis was made under isocratic conditions, with a mobile phase of acetonitrile:water (15:85, v/v). Resolution of all synthesis impurities was allowed. The method was also applied to stability studies of adosupine in solid state and in solution under different conditions. With the conditions used, only one degradation product was shown by HPLC analysis; it was isolated, characterized, and identified as the hydrolysis product of the lactam ring present in the adosupine structure.

Adosupine (10-acetylamino-5-methyl-5,6-dihydro-11[*H*]-dibenzo[*b*,*e*]azepin-6,11-dione) is a new potential drug under investigation for activity on urinary incontinence.[1-3] The unequivocal spectral characterization of adosupine and of all the other potential synthetic impurities, a reliable analytical method to identify and quantify all of them, and stability studies were all requested to support development of the product as a new drug. This paper summarizes the synthesis of the title compound, together with the characterization of and analytical studies performed on adosupine.

Results and Discussion

Synthesis and Spectral Characterization—*Synthesis*—The synthesis of adosupine is shown in Scheme 1. 1-Aminoanthraquinone (**1**) underwent a Schmidt reaction according to Caronna and Palazzo[4] to yield a mixture of the two isomers, 10- and 1-amino-5,6-dihydro-11[*H*]-dibenzo[*b*,*e*]azepin-6,11-dione (**2** and **3**).[5] The isomer **2** could be purified at this stage with repetitive crystallizations, but better total yields were obtained when the crude material was used in the following *N*-acetylation and *N*-methylation steps, purifying adosupine (**6**) from its isomer **7** at the very last stage. The structure of adosupine and related compounds were confirmed from their NMR and MS spectra.

^1H and ^{13}C NMR Spectra—The NMR spectra (Tables 1 and 2) were perfectly in accordance with the proposed structures. The downfield shift observed in $CDCl_3$ in **4** and **6** for H-9 and in **7** for H-2, when compared with DMSO-d_6, indicated that these hydrogen atoms were probably close to the carbonyl of the acetylamino group, in position 10 and 1, respectively, and just inside its deshielding zone. The carbonyl could be maintained in this position, when in $CDCl_3$, by an H bond between the NH of acetyalmino group and the carbonyl in position 11; however, in DMSO-d_6, the solvent could break H bonds, somehow freeing the acetylamino group. The absence of any relevant shift for H-9 in **2** is noteworthy because no acetylamino group was present in position 10. Similar reasons could also explain the slight downfield shift of H-1 in **2** and **4**.



Scheme 1—Synthesis of adosupine.

Mass Spectra—Electron impact mass spectra (Table 3) were consistent with the structural formulas of adosupine and related compounds. They all showed abundant molecular ions (base peaks in **2** and **4**) from which ions originate by losses indicating the substituent groups. To confirm the interpretation of some fragment ions in **6**, the mass spectrum of *N*-trideuteromethylated **6** was determined. This spectrum showed that there was no mass shift for the *m*/*z* 222 ion, thus confirming that it came from a loss involving the *N*-methyl group of the compound.

X-ray Analysis—Compounds **6** and **7** (Table 4 Figure 1) possessed very similar geometrical features: the nitrogen atoms of both structures showed sp^2 hybridization that was indicative of a large conjugation of the double bond in the N—C=O moieties. In both structures, the atoms of the seven-membered ring significantly diverted from their least-squares plane, conferring to the ring a barrel-like configuration; the O_2 and O_3 carbonyl oxygens and the C_{11} methyl carbon went in the opposite direction with respect to the phenyl groups, which formed with the seven-membered ring least-squares planes with angles of 31.9(1)° and 32.8(2)° in **6** and 27.4(5)° and 48.8(7)° in **7**. Finally, both structures showed a strong intramolecular N_1-H···O_2 hydrogen bond of 2.17(3) and 2.13(3) Å for **6** and **7**, respectively. The crystallization water in **7**, which lies on a twofold axis, links two symmetry-related molecules via a H-bond, with the O (H_2O)···O1 distance equal to 2.84Å.[3]

Analytical Study—A reversed-phase HPLC method for determination of adosupine and its potential impurities was developed on a stainless steel C18-CR (3 μm, 30 × 4.6 mm i.d.) LC-column (Perkin-Elmer). The mobile phase consisted of

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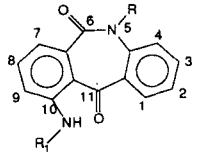
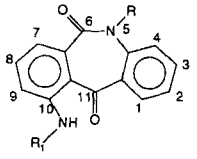
Table 1—^{1}H NMR Data of Adosupine and Related Compounds



Compound	^{1}H NMR, δ												
	H-1	H-2	H-3	H-4	H-7	H-8	H-9	H-10	R		NHR_1		$J_{Hi,Hj}$, Hz
2[a]	7.58 (m)	7.21 (m)	7.51 (m)	7.23 (m)	7.16 (m)	7.37 (m)	7.07 (d)	—	10.85 (b s) NH		6.7 (b s) NH_2	$J_{7,8}$ = 7.7	$J_{8,9}$ = 7.8
2[c]	7.74 (d)	7.19 (m)	7.46 (m)	6.99 (d)	7.56 (d)	7.37 (m)	6.93 (d)	—	8.40 (s,)NH		5.82 (b s)NH_2	$J_{1,2}$ = 7.7 $J_{7,8}$ = 7.7	$J_{3,4}$ = 7.9 $J_{8,9}$ = 7.8
4[a]	7.56 (m)	7.20 (m)	7.52 (m)	7.22 (d)	7.69 H7 + H9	7.92 (m)	7.69 H7 + H9	—	11.05 (s) NH	10.05 (s)NH	2.13 (s) CH_3	$J_{3,4}$ = 7.6	
4[c]	7.69 (d)	7.23 (m)	7.53 (m)	7.16 (m)	7.98 (d)	7.66 (t)	8.69 (d)	—	10.13 (s) NH	9.53 (s) NH	2.26 (s) CH_3	$J_{1,2}$ = 7.7 $J_{7,8}$ = 8.1	$J_{3,4}$ = 8.0 $J_{8,9}$ = 8.1
6[a]	7.54 (m)	7.32 (m)	7.59 (m)	7.52 (m)	7.67 (d)	7.79 (m)	7.76 (d)	—	3.54 (s) CH_3	9.85 (s) NH	2.04 (s) CH_3	$J_{7,8}$ = 7.9	
6[c]	7.44 (m)	7.21 (m)	7.51 (m)	7.26 (d)	7.78 (d)	7.55 (t)	8.57 (d)	—	3.57 (s) CH_3	9.85 (s) NH	2.22 (s) CH_3	$J_{3,4}$ = 7.0 $J_{8,9}$ = 8.1	$J_{7,8}$ = 8.1
7[a]	—	7.37 (d)	7.52 (m)	7.26 (d)	7.98 (m)		7.62–7.73 (m) H8 + H9	7.53 (m)	3.46 (s) CH_3	9.76 (s) NH	2.02 (s) CH_3	$J_{2,3}$ = 7.6	$J_{3,4}$ = 7.8
7[c]	—	8.13 (d)	7.41 (m)	7.03 (d)	8.07 (d)		7.58–7.64 (m) H8 + H9	7.55 (m)	3.51 (s) CH_3	9.42 (s) NH	2.17 (s) CH_3	$J_{2,3}$ = 8.4 $J_{7,8}$ = 6.7	$J_{3,4}$ = 8.1

[a] In hexadeuteriodimethyl sulfoxide. [b] —, Not applicable. [c] Letters in parentheses indicate the partical proton decoupling pattern.

Table 2—^{13}C NMR Data of Adosupine and Related Compounds



Compound	^{13}C NMR, δ															
	CH-1 (d)[c]	CH-2 (d)	CH-3 (d)	CH-4 (d)	CH-7 (d)	CH-8 (d)	CH-9 (d)	CH-10 (d)	CO-6 (s)	CO-11 (d)	R		R_1	other C(s)		
2[a]	131.0	127.9	134.8	120.7	125.7	134.8	122.3	—[b]	169.0	195.9	—[b]		—[b]	121.3	134.5	134.6
2[c,d]	132.3	122.4	137.2	121.5	126.7	135.2	122.4	—[b]	—[e]	—[e]	—[b]		—[b]	—[e]		
4[a]	132.1	125.3	134.5	121.4	133.6	129.7	131.1	—[b]	167.6	196.1	—[b]	170.6	25.1	132.8 136.1	133.9 136.8	134.3
4[b]	132.1	126.8	136.1	121.9	129.1	135.3	128.6	—[b]	169.5	198.1	—[b]	171.4	27.2	128.8 136.8	133.4 138.3	134.7
6[a]	129.9	127.2	134.1	124.2	133.9	130.2	130.4	—[b]	167.5	197.1	39.5	171.0	25.3	133.4 138.9	134.8(2C) 140.2	
6[c]	130.4	127.7	134.8	124.0	129.5	135.4	127.2	—[b]	168.7	199.3	40.0	171.3	27.1	129.0 139.5	134.7 141.0	136.6
7[a]	—[b]	122.0	133.5	124.6	132.6	133.9	134.1	129.0	167.5	196.9	40.3	170.6	25.0	131.0 142.2	132.1 143.2	136.3
7[c]	—[b]	121.5	134.8	120.7	133.7	134.6	135.1	128.8	168.3	199.2	41.3	171.0	26.9	127.6 143.3	131.9 143.7	137.7

[a] In hexadeuteriodimethylsulfoxide. [b] —, Not applicable. [c] In deuteriochloroform. [d] Assignments based on similarity with the other products and on their theoretical values. [e] Product too poorly soluble in deuteriochloroform to identify all carbon atoms in a reasonable lapse of time.

acetonitrile:water (15:85), the flow rate was set at 2.0 mL/min, the injection volume was 3 μL, and the oven temperature was set at 50 °C. The wavelength selected for detection was 234 nm with a bandwidth of 8 nm (reference wavelength 550 nm, with a bandwidth of 100 nm). This wavelength allowed a good separation of adosupine and all byproducts of its synthesis (Figure 2) in a very short time. Although baseline separation could not be achieved for 2 and 3, the accuracy and precision of dosage of these potential impurities were considered satisfactory (see *Validation of HPLC Method for Potential Synthesis Impurities Assay*), and no further attempt was made to improve separation. The same analytical conditions were applied in the study of the stability of adosupine bulk material.

Validation of HPLC Method for Adosupine assay—The response linearity of the method was tested by injecting five solutions (500–1500 μg/mL) of adosupine in acetonitrile, two times; by plotting the peak areas against concentration, a linear regression (y = 1663.538x + 17.282) was obtained, with a determination coefficient (r^2) of 1.000. The intercept value was not different from zero (*t* test). The chromatographic precision

Table 3—MS Data of Adosupine and Related Compounds[a]

m/z	2		4		6		7	
294	—[b]		—		M	(64.3)	M	(94.9)
293	—		—		M-H.	(5.5)	M-H.	(27.9)
280	—		M	(100)	—		—	
279	—		—		M-CH_3.	(2.9)	—	
266	—		—		M-CO	(2.9)	M-CO	(4.5)
265	—		M-CH_3.	(4.9)	M-CO-H.	(1.5)	M-CO-H.	(5.1)
252	—		M-CO	(2.1)	M-CH_2CO	(43.9)	M-CH_2CO	(46.4)
251	—		M-CO-H.	(4.3)	M-CH_3CO.	(100)	M-CH_3CO.	(100)
238	M	(100)	M-CH_2CO	(87.0)	—		—	
237	M-H.	(59.5)	M-CH_3CO.	(85.1)	—		—	
236	—		—		252-NH_2.	(44.4)	252-NH_2.	(11.6)
224	—		—		252-CO	(41.7)	252-CO	(67.5)
223	—		—		251-CO	(27.1)	251-CO	(18.5)
222	M-NH_2.	(40.9)	238-NH_2.	(71.2)	251-NCH_3	(22.1)	251-NCH_3	(3.3)
210	M-CO	(47.7)	238-CO	(92.8)	—		—	
209	210-H.	(29.4)	210-H.	(39.2)	224-CH_3.	(15.4)	224-CH_3.	(13.3)
208	—		—		236-CO	(10.2)	236-CO	(6.9)
207	—		—		208-H.	(7.2)	208-H.	(8.2)

[a] The numbers in parentheses indicate percent abundances of the ions. The periods after some data indicate radical loss. [b] —, Not applicable.

Table 4—Crystal and Refinement Data for 6 and 7

Parameter	6	7
Molecular formula	$C_{17}H_{14}N_2O_3$	$C_{17}H_{14}N_2O_3 \cdot 0.5\ H_2O$
Molecular weight	294.31	303.32
a, Å	4.622 (1)	11.403 (7)
b, Å	9.299 (1)	34.433 (8)
c, Å	16.752 (1)	7.422 (5)
α, deg	86.402 (4)	90
β, deg	86.052 (6)	90
γ, deg	79.090 (6)	90
V, A^3	704.4 (2)	2915 (3)
z	2	8
Space group	P1	Aba2
d_{calc}, g × cm^{-3}	1.39	1.38
Radiation	Cu-Kα	Mo-Kα
Temp., °C	25	25
μ, cm^{-1}	7.53	0.62
R^a	0.043	0.122
$R_w{}^b$	0.041	0.083

[a] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w(F_o)^2]^{1/2}$.

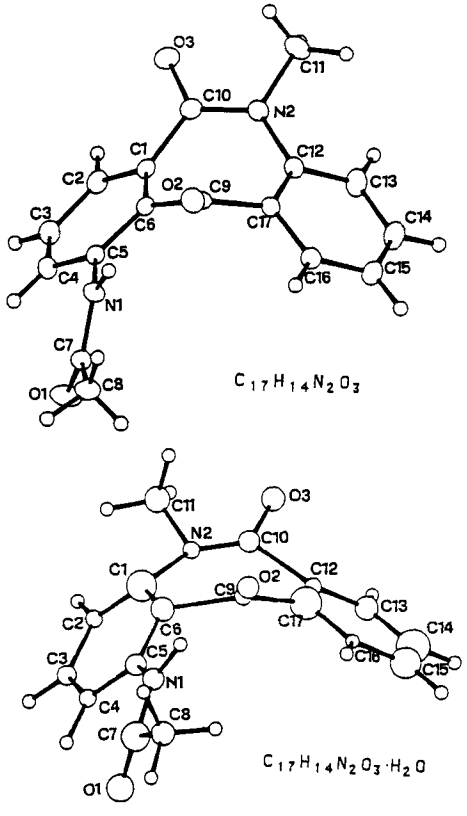


Figure 1—ORTEP drawing of **6** and **7**.

expressed as coefficient of variation (CV) was calculated by injecting five replicates of a solution of adosupine at 1000 μg mL. The CV was <0.1%.

Validation of HPLC Method for Potential Synthesis Impurities Assay—To test the response linearity, four solutions of adosupine at 1000 μg/mL, containing scalar amounts of each impurity as listed in Table 5, were prepared. Linear regression curves were obtained for each one by injecting each solution twice and measuring peak areas. The equation and determination coefficient for each impurity are listed in Table 6. In all cases, the intercept value was not statistically different from zero. The chromatographic precisions expressed as CV were calculated by injecting five replicates of solutions 2 and 4, corresponding to the maximum amount accepted for each impurity and to a quarter of this value, respectively (Table 7).

Accuracy and precision of the assay for the determination of the potential impurities were measured as follows: five solutions of adosupine (1000 μg/mL), containing known amounts of each impurity, were injected twice, and the amounts of impurities were calculated against a standard solution of impurities at equal concentrations. Accuracy was expressed as a percent ratio of the found values to the added ones, and precision was expressed as CV times *t* (from *t* test) for the appropriate degrees of freedom (see Table 8). The sensitivity of the assay was expressed as minimum detectable level (MDL) and minimum quantifiable level (MQL), which are defined as follows: MDL = 3 × noise/response factor; and MQL = 10 × noise/response factor (noise is calculated from the regression analysis as the square root of the mean square residual, and response factor is the slope of the regression curve). The limits of detection and the lowest quantifiable levels are listed in Table 8.

Polymorphism—Adosupine was subjected to crystallization from various solvents under different conditions. The crystals obtained were observed by a polarizing microscope and analyzed by their mp, IR spectra, and differential scanning calorimetry

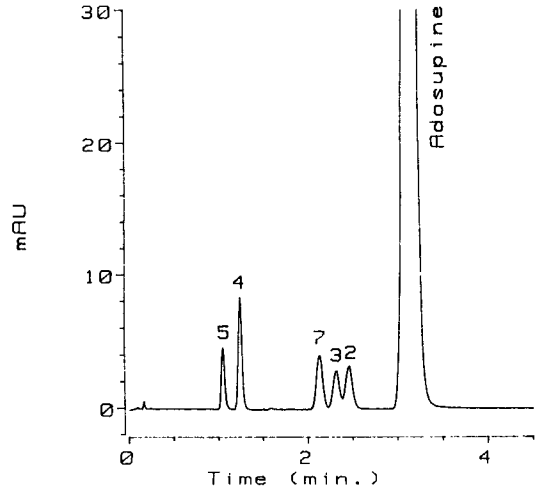


Figure 2—A typical chromatogram obtained from a 1000-μg/mL solution of adosupine in acetonitrile spiked with 1% of each potential synthesis impurity.

Table 5—Percent Composition of Synthesis Impurities of Adosupine in the Solutions Injected for Response Linearity Testing

	Composition, %			
Product	Solution 1	Solution 2	Solution 3	Solution 4
2	1.13	0.56	0.28	0.14
3	0.92	0.46	0.23	0.12
4	1.91	0.95	0.48	0.24
5	0.99	0.49	0.25	0.12
7	4.24	2.37	1.43	0.97

Table 6—Linear Regression Equations and Determination Coefficients of Synthesis Impurities of Adosupine

Product	Equation	Determination Coefficient
2	y = 53.116x + 1.763	0.998
3	y = 52.342x + 1.437	0.999
4	y = 54.033x + 0.225	1.000
5	y = 47.014x + 0.587	1.000
7	y = 48.445x + 4.868	1.000

Table 7—Coefficients of Variation of Peak Areas of Synthesis Impurities of Adosupine After Five Replicate Injections

	CV, %	
Product	Solution 2	Solution 4
2	2.72	3.08
3	2.68	2.99
4	0.44	1.29
5	0.89	2.46
7	0.21	0.28

(DSC) traces. Only one crystalline form was always observed; evaporation with a stream of nitrogen from a dichloromethane solution at room temperature yielded an amorphous solid. Two forms were observed for positional isomer **7**: the main form was obtained from ethanol and melted at 170 °C, and another crystalline form was obtained from water and melted at 140 °C. The two forms also were clearly detected with DSC analysis and probably differ in their relative contents in water.

Stability Studies[6]—The stability of adosupine raw material was tested as follows: (*1*) first, the solid substance at different temperatures and illumination conditions was studied. One lot of adosupine was held at 50 °C, one at 40 °C, and one at room temperature (rt); the latter were studied both in the dark and in ambient light. These samples were analyzed by HPLC under the above conditions, using as a standard a sample of the same lot of raw material held at 0 °C under a nitrogen atmosphere. This study was conducted for 2 years (Figure 3). The product was found to be perfectly stable, without showing significant titer decrease nor appearance of impurities. (*2*) Next, the solid substance of four different lots at RT were studied. These samples had been held at ambient temperature for periods ranging from 27 to 60 months. For each one, HPLC analysis, loss on drying at 105 °C, and IR spectra were used, and no meaningful variation with respect to first analysis was registered. (*3*) Finally, suspensions in aqueous buffers at different pH or in aqueous solutions of reducing or oxidizing reagents were studied. One lot of adosupine was suspended in 0.1 M buffers at pH 2.0, 7.0, and 12.0, and in solutions of sodium hydrogen sulfite (5% w/v) and hydrogen peroxide (5% w/v); at chosen times, the samples were dissolved in acetonitrile and analyzed by HPLC. This study was conducted for 320 days (Figure 4). Adosupine was stable at pH 2.0 and in solution of sodium hydrogen sulfite and hydrogen peroxide. In buffer solution at pH 7.0 and 12.0, adosupine was degraded at a rate depending on the pH, essentially giving a unique product (Figure 5). This degradation product was isolated by extraction with methyl–ethyl ketone, purified by crystallization from benzene, and characterized by ^1H and ^{13}C NMR, and IR and MS spectroscopy (see *Experimental Section*). All spectroscopic data, as well as polarity considerations based on HPLC retention time, were in agreement with a structure in which the azepindione ring of adosupine was hydrolyzed; in particular, the wide band at 3250 cm^{-1} and the one at 1720 cm^{-1} are absent in related compounds and are characteristic of a carboxy group. The mass spectrum indicates a probable molecular mass of 312 amu and a loss of water (ion

Table 8—Accuracy, Precision, MDL, and MQL of the Method for Potential Synthesis Impurities Assay

Product	Accuracy	Precision, %	MDL, %	MQL, %
2	98.67 ± 2.94	8.9	0.043	0.14
3	100.21 ± 1.83	5.5	0.029	0.10
4	99.96 ± 1.48	4.4	0.018	0.06
5	101.14 ± 1.26	3.7	0.016	0.05
7	99.79 ± 1.54	4.6	0.025	0.08

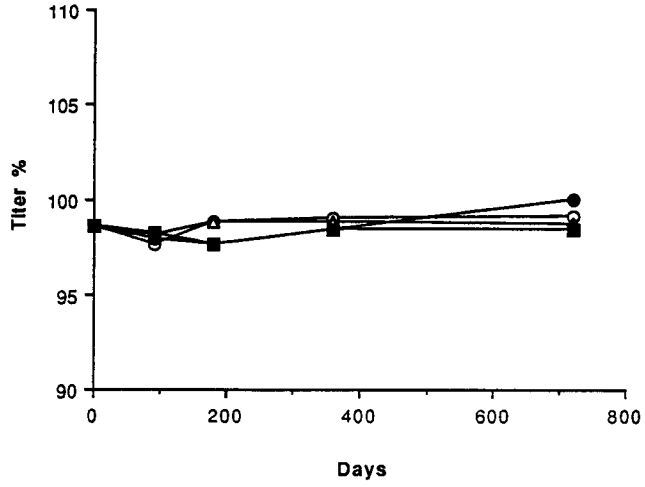


Figure 3—HPLC titer variation versus time for adosupine as a solid substance under different conditions. Key: (O) 40 °C; (●) 50 °C; (Δ) R.T. (dark); (■) R.T. (light).

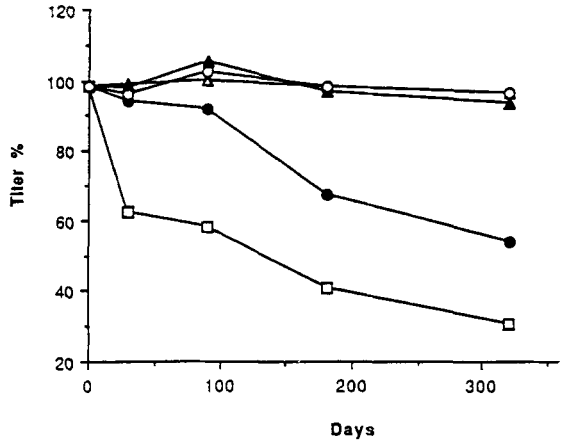
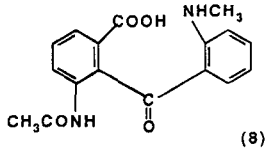


Figure 4—HPLC titer variation versus time for adosupine as a suspension under different conditions. Key: (△) pH 2; (●) pH 7; (□) pH 12; (▲) H_2O_2; (○) $NaHSO_3$.

m/z 294) and a COOH radical (*m/z* 367) from the molecular ion; thus, the degradation product of adosupine in neutral-alkaline suspension is 2-(2-methylaminobenzoyl)-3-acetylamino-benzoic acid (8, see structure).



(8)

Experimental Section

Equipment—All NMR spectra were recorded on a Varian Gemini-200 spectrometer. The APT, COSY, and HETCOR experiments,[7-10] in addition to ^1H and ^{13}C NMR spectra, were performed with standard Varian software (version 6.2). The mass spectra were recorded on a Hewlett-Packard 5988A MS in the electron-impact mode at 70 eV. Samples were introduced by a direct inlet probe that was ballistically heated to 250 °C. The IR spectra were recorded on a Perkin Elmer 1710 Fourier transform IR spectrometer. HPLC studies were performed on a Hewlett-Packard 1090 M liquid chromatograph equipped with a variable wavelength diode array detector, a variable volume autoinjector, an autosampler, and a thermostatic column oven, and interfaced with a work station. The DSC analyses were performed on a Perkin Elmer DSC 7 with Thermal Analysis Controller TAC 7/DX.

Synthesis of 10-Acetylamino-5-methyl-5,6-dihydro-11[*H*]-dibenzo[*b,e*]azepin-6,11-dione (Adosupine; 6)—1-Aminoanthraquinone (0.3 mol) was slowly dissolved in 400 mL of H_2SO_4, with stirring and at temperatures <30 °C. Then, 0.4 mol of sodium azide were slowly added to the solution, with stirring, over a period of 2 h while keeping the temperature <30 °C with the aid of an ice bath. After an additional 2 h of stirring at room temperature, the mixture was poured into 6 kg of crushed ice and filtered with suction. The solid was resuspended into 10 L of cold water, neutralized with sodium bicarbonate, and filtered again to yield 80% as a mixture of **2** and **3** (in proportion of ~65:35%); mp, 250–265 °C. Pure isomer **2** could be obtained with repetitive recrystallizations from dioxane; mp, 273–275 °C; IR (KBr, cm^{-1}): 3483, 3368, 3181, 3036, 1626, 1601, and 1893.

Next, 0.2 mol of amino-5,6-dihydro-11*H*-dibenzo[*b,e*]azepin-6,11-dione, as a mixture of isomers **2** and **3**, was suspended in 450 mL of a solution of THF and acetic acid (2:1) and treated with 150 mL of acetic anhydride. The mixture was kept at 70 °C for 8 h while stirring, cooled to room temperature, and poured into 5 L of cold water. The solid was filtered and dried to yield

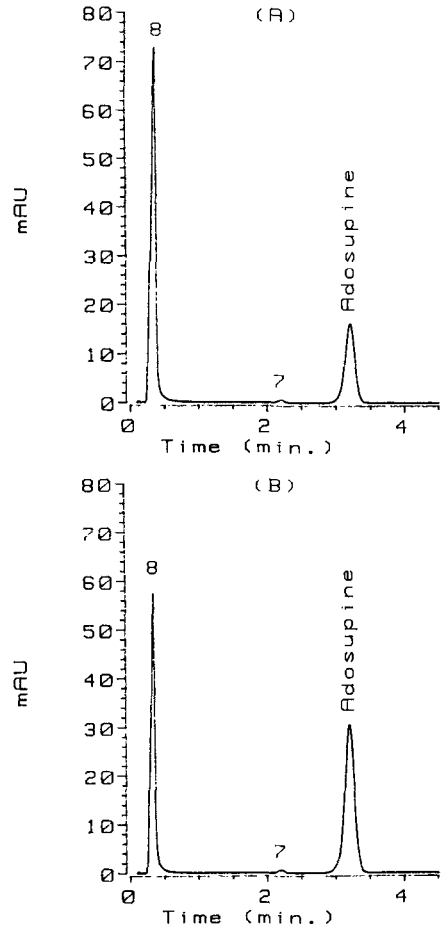


Figure 5—Chromatograms obtained by analysing adosupine suspensions held at (**A**) pH 12.0 and (**B**) pH 7.0 for 320 days. Compound **7** is present as an impurity in the raw material used for the test and is not generated during stability testing.

90% as a mixture of **4** and **5** (in proportion of ~65:35%), mp, 260–263 °C. Pure **4** could be obtained starting from pure **2** or recrystallizing the mixture of **4** and **5** several times from ethanol; mp, 272–273 °C; IR (KBr, cm^{-1}): 3325, 3174, 3041, 1695, 1664, 1640, and 1605.

Anal.—Calcd for C, 68.57; H, 4.321; N, 9.99; found: C, 68.30; H, 4.36; N, 9.78.

Acetylamino-5,6-dihydro-11*H*-dibenzo[*b,e*]azepin-6,11-dione (0.15 mol) as a mixture of isomers **4** and **5**, was dissolved in 300 mL of DMF with stirring, treated with 0.16 mol NaH (80%) and, after 1 h, treated with 0.25 mol methyl iodide. The solution was kept at room temperature for an additional 48 h, poured into 5 L of water, and filtered. The isomeric mixture (~70:30%) was recrystallized from ethanol several times obtaining pure adosupine (**6**) in a yield of 35% as pure isomer; mp, 203–205 °C; IR (KBr, cm^{-1}): 3324, 1665, 1642, and 1593.

Anal. Calcd for C, 69.38; H, 4.79; N, 9.52; found: C, 68.98; H, 4.76; N 9.29.

The water solution was concentrated to yield a white product that was determined to be isomer **7**; yield, 5%; mp, 163–165 °C; IR (KBr, cm^{-1}): 3511, 3365, 3069, 1689, 1660, 1636, and 1580. A mixture containing the two isomers in a proportion of ~50:50% was recovered by evaporation of the ethanol solutions in a yield of 48%. No further purification of any of the two isomers could be accomplished by repetitive recrystallization of this mixture with ethanol or comparable solvents.

Synthesis of 10-Acetylamino-5-trideuteromethyl-5,6-dihydro-11[*H*]-dibenzo[*b,e*]azepin-6,11-dione (*N*-Trideuteromethylated Adosupine)—First, 0.03 μmol of acetylamino-

5,6-dihydro-11*H*-dibenzo[b,e]azepin-6,11-dione (**4**) was dissolved in 5 mL of DMF with stirring, treated with 0.03 μmol NaH (80%) and, after 1 h, treated with 0.25 mol trideuteromethyl iodide. The solution was kept at room temperature for an additional 48 h, poured into 100 mL of water, and filtered. The solid material was recrystallized from dioxane and characterized by its mass spectrum; MS (amu): 297 (M^+), 255 ($M^+ - CH_2CO$), 254 ($M^+ - CH_3CO$), 239 (255 – NH_2), 227 (255 – CO), 226 (254 – CO), and 222 (254 – NCD_3).

Synthesis of 2-(2-Methylaminobenzoyl)-3-acetylaminobenzoic Acid—Adosupine (2 mM, **6**) was suspended in a 1 N NaOH solution and stirred until a limpid yellow solution was achieved. The solution was acidified with a 37% solution of HCl (pH ~5) and extracted with methyl-ethyl-ketone until the aqueous solution became clear. The organic layer was dried with anhydrous sodium sulfate and evaporated to dryness. The oily product obtained was crystallized from benzene, and the yellow solid was collected by filtration (yield, ~25%). The product melted with decomposition at ~170 °C; ^{1}H NMR (DMSO-d_6, ppm): 1.8 (s, CH_3CO), 2.9 (d, CH_3-NH), 3.4 (s, aminic NH), 6.3–7.9 (m, 7 H, aromatic), 8.6 (s, 1 H, amidic NH), 9.3 (s, 1 H, COOH); ^{13}C NMR (DMSO, ppm): 22.8 ($COCH_3$), 29.1 (CH_3-NH), 167.1 (COOH), 169.3 ($COCH_3$); IR (cm^{-1}): 3344 (NH), 3250 wide (carboxilic OH), 1720 (carboxylic CO), 1700 (amidic CO), 1620 (ketonic CO); MS (amu): 312 (M^+), 294 ($M^+ - H_2O$), 269 ($M^+ - CH_3CO$), 268 ($M^+ - CO_2$), 267 (M^+-COOH).

X-ray Crystal Structure Determination—Single crystals of **6** and **7** were mounted on an Enraf-Nonius CAD4 X-ray diffractometer. A summary of the crystallographic data is reported in Table 3.[11] Unit cell parameters were determined from angular settings of 25 carefully centered reflections for both compounds. A total of 2480 and 1460 reflections were collected for **6** and **7**, respectively. Intensities were then corrected for Lorentz and polarization effects. A total of 2245 and 331 reflections with $I > 3\sigma(I)$ were used in the structure determination of **6** and **7** respectively. Both structures were solved by direct methods of MULTAN,[12] which showed all non-hydrogen atoms. Refinements were then performed by means of the full-matrix least-squares program SHELX76.[13] Anisotropic temperature factors were used for all non-hydrogen atoms of **6**, whereas the hydrogen atoms were refined isotropically. For **7**, because of the low number of observed reflections and their poor quality, all atoms were refined isotropically, with calculated position and an overall temperature factor (U) of 0.08 A^2 for the hydrogen atoms. In **7**, the carbon atoms of the two phenyl groups were refined as rigid groups.

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