(m, 1 H), 5.8 (dd, 1 H, J = 5 and 3 Hz), 6.16 (dd, 1 H, J = 5 and 3 Hz), 7.23 (d, 2 H, J = 9 Hz), 7.76 (d, 2 H, J = 9 Hz), 7.7 (masked, 1 H); MS m/e 305 (8), 303 (20), 151 (100), 149 (100), 107 (52), 105 (30), 91 (40), 79 (42).

Anal. Calcd for $C_{16}H_{20}N_2O_2S$: C, 63.13; H, 6.62; N, 9.20; S, 10.53. Found: C, 63.28; H, 6.68; N, 9.16; S, 10.60.

7,7-Dimethylnorbornadiene (1). A solution of tosylhydrazone **12** (1.2 g, 3.94 mmol) in 10 mL of dry THF was added slowly at −78 °C over a period of 10 min to a stirred solution of lithium diisopropylamide (prepared from diisopropylamine (1.62 g, 16 mmol) and 5.4 mL of a 1.85 M solution of n-BuLi in hexane) in 15 mL of dry THF. Stirring was maintained for 10 min at −78 °C, and the temperature was allowed to rise to room temperature overnight. The brown mixture was diluted with 150 mL of pentane and washed with brine (4 × 50 mL), then with a 1 M solution of NaH_2PO_4 (2 × 30 mL), and finally with brine again (2 × 30 mL). Drying over Na_2SO_4 followed by distillation of most of the solvent (40–70 °C) gave a concentrated solution which was shown by GLC (10% SE 30/Chromosorb W, 56 °C, N_2 1.4 kg/cm², 3 m × 3 mm, Carlo Erba chromatograph) to be a clean mixture of 7,7-dimethylnorbornadiene (**1**) (70%, retention time 5.7 min) and 7,7-dimethylnorbornene (**13**) (30%, retention time 6.7 min). Purification and isolation was achieved by preparative GLC (15% SE 30/Chromosorb W, 124 °C, N_2 100 mL/min, 3 m × 8 mm, Perkin-Elmer 990 chromatograph) to give **1** as a colorless waxy solid (127 mg, 27%) and **13** (40 mg, 8%).[3c] The yield of **1** before GLC separation was estimated to be 43% by GLC calibration. Samples not stored in ampules volatilized rapidly even at 0 °C.

7,7-Dimethylnorbornadiene (1): ^{1}H NMR ($CDCl_3$, 100 MHz)[24] δ 1.12 (s, 6 H), 3.05 (m, 2 H), 6.58 (t, J = 2 Hz, 4 H); ^{13}C NMR ($CDCl_3$)[24] (δ from Me_4Si) 24.26 (s, C-8,9), 59.99 (s, C-1,4), 84.83 (s, C-7), 142.44 (s, C-2,3,5,6); mass spectrum, m/e 120 (10), 105 (100). Exact mass: calcd 120.09389; found 120.09270.

7,7-Dimethylnorbornene:[3c] ^{1}H NMR ($CDCl_3$) δ 0.90 (s, 3 H), 0.95 (s, 3 H), 1.5–2.2 (m, 4 H), 2.25 (m, 2 H), 5.95 (t, J = 2 Hz, 2 H).

Registry No.—**1**, 68757-94-8; **2**, 4125-18-2; **4**, 54639-78-0; **5**, 22748-16-9; **6**, 68757-95-9; **7**, 68757-96-0; **8**, 68757-97-1; **9**, 68757-98-2; **10**, 68757-99-3; **11**, 68758-00-9; **12**, 68758-01-0; **13**, 6541-60-2; maleic anhydride, 108-31-6; α-chloroacrylonitrile, 920-37-6.

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(24) ^{1}H and ^{13}C NMR data are in good correlation with those of norbornadiene itself [^{1}H NMR ($CDCl_3$) δ 2.00 (t, 2 H), 3.58 (m, 2 H), 6.75 (t, 4 H); ^{13}C NMR ($CDCl_3$) δ 50.9 (C-1,4), 75.4 (C-7), 143.4 (C-2,3,5,6)] or 1,7,7-trimethyl-2,5-norbornadiene [^{1}H NMR ($CDCl_3$) δ 1.02 (s, 6 H), 1.19 (s, 3 H), 3.03 (m, 1 H), 6.5 (m, 4 H)].
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On a New Azatetracycline Ring System

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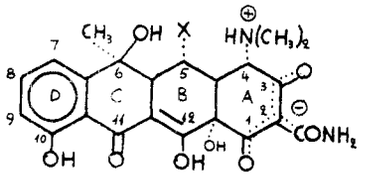
The structures of some substances having a novel azatetracycline ring system were determined. The azatetracycline ring system was produced by a rearrangement of the tetracycline ring system.

During a study of the transformations of some substances of the tetracycline group, we have found an unusual reaction which, via a molecular rearrangement, leads to a novel azatetracycline ring system.[1] Thus, when 11a-chloro-5-hydroxytetracycline 6,12-hemiketal (**2a**),[2] which is a derivative of 5-oxytetracycline (**1b**), was suspended in its amphoteric form in pyridine and warmed gently, it was observed that shortly after dissolution a crystalline yellow substance precipitated. We call this substance cyclazoxytetracycline (COT). Determination of its structure as **7** is detailed herein.



1a X = H
1b X = OH

Although other ring systems are derived from the tetracycline one, in order to facilitate the understanding of the transformations we will in general identify throughout the article the different functional groups or rings by the usual nomenclature in this field, i.e., by the number or letter of the position occupied on the original tetracycline ring system (**1**).

The analysis of cyclazoxytetracycline indicated that its elemental composition was the same as that of the starting

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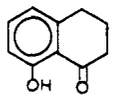
Table I. UV Spectra, nm (ε)

11a-chloro hemiketal **2a**[a]		267 (24 000)			341 (4800)
lactone **17**[a]		269 (24 400)			352 (3600)
CDOT (**19**)[a]		273 (25 900)			360 (4100)
COT (**7**)[a]		271 (24 800)			352 (4440)
8-hydroxy-1-tetralone (**10**)[b,c]		261 (9330)			335 (3090)
carboxamidodimedone (**11**)[a,d]		258 (17 700)			
terranaphthol (**12**)[c,e]	232 (61 660)		312 (7760)	327 (7760)	341 (7760)
ACOT (**8**)[a]	234 (64 000)	264 (20 000)	316 (8000)	330 (10 200)	345 (11 400)

[a] MeOH–0.01 N HCl. [b] Acid ethanol. [c] Reference 3. [d] Reference 4. [e] Ethanol.

hemiketal **2a**, that is $C_{22}H_{23}ClN_2O_9$. Therefore, it must have been formed by a molecular rearrangement, without gain or loss of atoms.

Its UV spectrum in acidic methanol is very similar to that of hemiketal **2a** and to the sum of the spectra of 8-hydroxytetralone (**10**)[3] and carboxamidodimedone (**11**),[4] which are



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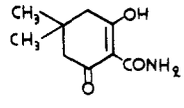

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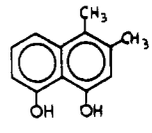

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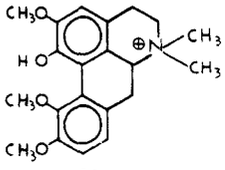

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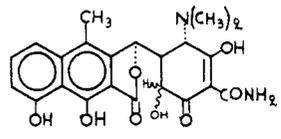


14

models for the C, D and the A ring chromophores of the hemiketal **2a**, respectively. In Table I some data on maxima are given. The data indicate that these chromophores, present in the starting hemiketal **2a**, were not destroyed after the rearrangement.

In the IR spectrum, a strong band at 5.62 μm indicated the presence of a five-membered aliphatic lactone,[2,5a] which must have been formed with the 12-carbonyl group since otherwise the UV chromophoric systems would have been changed.

One important clue to the type of reactions leading to COT was the startling observation that its chlorine atom was no longer bound to C(11a) by a covalent bound, as it was in the original hemiketal **2a**, but was in fact ionic. This chloride anion could be easily removed by adjusting the pH of an aqueous suspension of solid COT to 4.0, filtering, and washing the solid with water until chloride was absent from the washings. In this way, an organic substance free of chlorine was obtained. From this substance, the original COT could be regenerated again by treatment with diluted aqueous HCl. This means that COT was actually a hydrochloride or a chloride, an unusual result considering that the product crystallized from pyridine.[6]

When 11a-bromo-5-hydroxytetracycline 6,12-hemiketal (**2c**)[7] was subjected to the same reaction conditions in pyridine, COT hydrobromide was obtained, which was converted into the amphoteric form containing no halogen and finally into COT hydrochloride by treatment with aqueous HCl. The COT hydrochloride so obtained was identical with that obtained directly from 11a-chloro hemiketal (**2a**).

The ^{1}H NMR spectra of COT afforded the second fundamental hint concerning its structure. In TFA, the dimethylamino group, instead of giving a pair of neighboring doublets or a singlet at δ 3.3–3.5 as is usual,[8] gave two well-separated singlets at δ 3.78 and 4.21, respectively (see Table II). In a basic solvent, such as pyridine-d_5, instead of giving only one singlet upfield, usually at δ 2.5–2.7,[8] it still showed two well-separated singlets, which, on the contrary, even moved slightly downfield to δ 3.94 and 4.29.

This unequivocally indicated that the dimethylamino group was now quaternary, forming part of a cyclic structure in which the two methyl groups are differentiated due to distinct magnetic environments. As a comparison reference, the ^{1}H NMR spectra of the quaternary aporphine alkaloid *N*-methylcorydine (**13**)[9] were made and the two signals of the *N*-methyls in each of the solvents, TFA and pyridine-d_5, are given in Table II.

Analyzing these experimental facts, it becomes evident that the 4-dimethylamino group must have reacted with the chlorine-bearing C(11a) by an intramolecular nucleophilic substitution reaction, giving rise to a quaternary dimethylammonium group and changing the chlorine atom from the covalently bounded condition to the ionic state.

For such a reaction to occur, it is obvious that there must be a prior lactone formation involving the 12-carbonyl group and the 5- or 6-hydroxyl group, with cleavage of either the C(11a)–C(12) or the C(12)–C(12a) bond. With the assistance of molecular models, it is evident that the only lactone that allows the reaction of the free pair of electrons of the 4-dimethylamino group with the C(11a) is that formed by the interaction of the 5-hydroxyl group with the 12-carbonyl group and cleavage of the C(11a)–C(12) bond. This leads to **7** as the probable structure of COT. This structure was confirmed by further chemical evidence.

When COT was subjected to a strongly acidic aqueous medium, it lost the elements of water, giving anhydrocyclazoxytetracycline (ACOT) (**8**). Its UV spectrum in acidic methanol is very similar to the sum of those of carboxamidodimedone (**11**)[4] and terranaphthol (**12**)[3] (see Table I), confirming that rings A and C, D of hemiketal **2a** were not destroyed. Likewise, the absence of absorption peaks at wavelengths higher than 345 nm, especially in the region of 375–377 nm, characteristic of apoterramycins (**14**),[3] also shows that the original C(12), now forming a lactone, must have remained linked to the 12a position and not to the 11a. This is further confirmed by the IR spectrum of ACOT since it still presents unaltered the same strong aliphatic lactone band at 5.62 μm.[10]

On the ^{1}H NMR spectra of ACOT (**8**), the following comments can be made. (a) The C(6)-CH_3 peak shows the usual downfield shifts associated with the aromatization of ring C:[8] to δ 2.78 in TFA and δ 2.44 in pyridine-d_5 (see Table II). (b) The dimethylamino group, due to its cyclic quaternary state, continues to give two well-separated singlets, both in TFA and pyridine-d_5, but now it also shows notable downfield shifts, confirming its bonding to the aromatized C ring (see Table II). (c) In the aromatic region only three hydrogens are detected between δ 7.15 and 7.95, showing a characteristic ABC splitting pattern similar to that produced by the 7, 8, and 9 hydrogens of other substances of the tetracycline family. (d) In addition to the described peaks, the only other signals of the ^{1}H NMR spectrum in TFA correspond to three aliphatic hydrogens, in accordance with structure **8**.

When COT chloride was reacted with P_2O_5 in DMF, a

Table II. ^{1}H NMR Data, δ

	$N(CH_3)_2$		C-CH_3	
	TFA[a]	py-d_5[b]	TFA[a]	py-d_5[b]
COT (**7**)	3.78 (s) 4.21 (s)	3.94 (s) 4.29 (s)	2.09 (s)	1.96 (s)
CDOT (**19**)	3.84 (s) 4.20 (s)	3.83 (s) 4.23 (s)	1.77 (d, J = 5.5 Hz)	1.63 (d, J = 6 Hz)
ACOT (**8**)	4.20 (s) 4.60 (s)	4.28 (s) 4.73 (s)	2.78 (s)	2.44 (s)
lactone **21**	3.16 (s) 3.35 (s)	2.82 (s)		
lactone **17**	3.29 (s)	2.78 (s)	1.62 (d, J = 7.5 Hz)	1.70 (d, J = 7.5 Hz)
OTC (**1b**)	3.27 (s)	3.07 (s)	2.66 (s)	2.26 (s)
TC (**1a**)	3.30 (d, J = 5 Hz) 3.39 (d, J = 5 Hz)		2.45 (s)	
N-methylcorydine (**13**)	3.19 (s) 3.58 (s)	3.33 (s) 3.80 (s)		

[a] TFA = trifluoroacetic acid. [b] Py-d_5 = pyridine-d_5.

zwitterionic nitrile (**9**) was obtained. The IR spectrum of **9** showed strong bands at 4.56 μm, which indicates the presence of an unsaturated nitrile group,[5b,c] and at 5.62 μm, due to the five-membered saturated lactone.[5a]

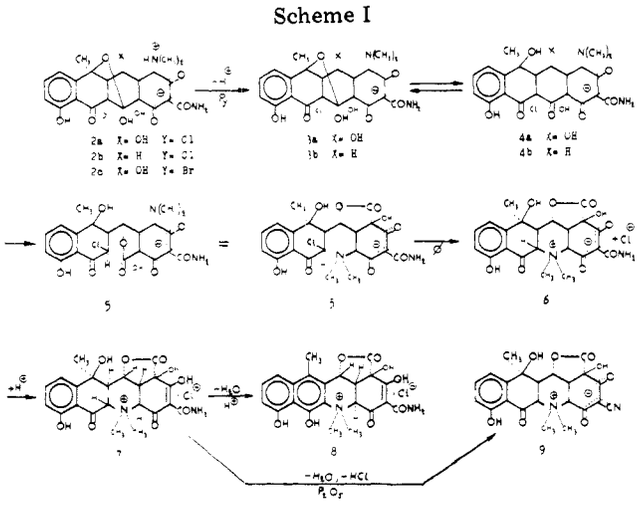
Having concluded from the above added evidence that the structure of COT hydrochloride is indeed **7**, it was reasonable to assume that the most probable chemical way leading from hemiketal **2a** to COT (**7**) is that shown in Scheme I.

It is known that 11a-chloro hemiketals of the type of **2a** in solution exist in part as the open keto form similar to **4a**.[2] It is also known that 11a-halotetracyclines possessing a 12-carbonyl and a 5-hydroxyl group, upon increasing the pH, may decompose by scission of the C(11a)–C(12) bond, giving lactones of the type **5**,[2] presumably with inversion of the configuration of carbon 11a.

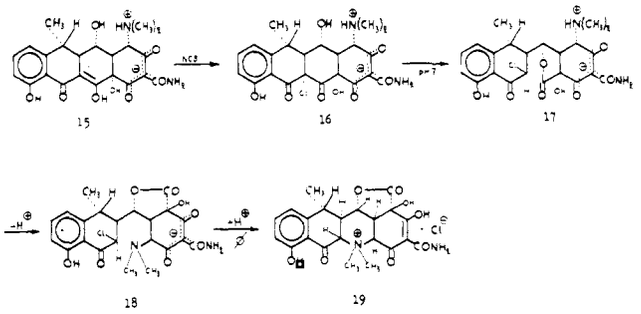
From a theoretical point of view, the lactone **5** should have little difficulty in cyclizing, by means of an intramolecular nucleophilic substitution reaction, to a 12-azatetracycline ring system since the N(4) can approach C(11a) from the opposite side of the chlorine in almost the optimum direction, being sterically guided by the rest of the molecule.

A few limited attempts to isolate the inferred intermediate lactone **5** were unsuccessful. This was partly due to its probable easy cyclization (as will be shown with similar substances) and to the complexity of the reaction mixture since hemiketal **2a** may also undergo decomposition by other pathways, *especially in basic media*.[11]

Scheme I



Scheme II



As expected, when 11a-chlorotetracycline 6,12-hemiketal (**2b**)[2] was subjected to the same reaction conditions employed for preparing COT from **2a**, no similar substance was obtained since **2b** did not have the necessary 5-hydroxyl group to form the γ-lactone.

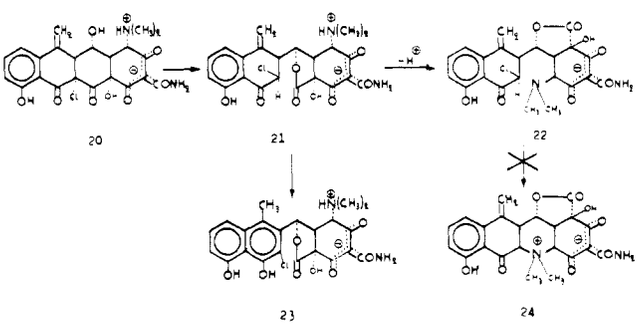
On the other hand, the situation is different in the case of α-6-deoxy-5-hydroxytetracycline (**15**). This substance lacks the 6-hydroxyl group and therefore cannot form 6,12-hemiketals, but it possesses the critical 5-hydroxyl group.

Reacting **15** with *N*-chlorosuccinimide transformed it into 11a-chloro-α-6-deoxy-5-hydroxytetracycline (**16**) (see Scheme II). Without isolating this substance at the end of the reaction, it was converted into the lactone **17**, diluting the reaction mixture with water and adjusting the pH to 7.

As expected, the lactone **17** cyclized straightforwardly to **19**, a substance we call cyclazo-α-6-deoxy-5-oxytetracycline (CDOT). This cyclization could be induced easily by warming **17** in a large variety of solvents, such as acetic acid, nitromethane, pyridine, ethanol, etc.

The UV spectra of CDOT were similar to COT and also

Scheme III

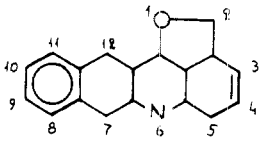


showed the presence of the intact 8-hydroxytetralone and ring A chromophores (see Table I). The IR spectrum showed the aliphatic γ-lactone carbonyl peak at 5.60 μm, and the ^{1}H NMR spectra gave *N*-methyl signals similar to COT, which also showed that the dimethylamino group was now quaternary and forming part of a cyclic structure (see Table II).

It was assumed, likewise, that it should also be possible to effect the cyclization of lactone **21** (see Scheme III), obtained from 11a-chloro-6-demethyl-6-deoxy-6-methylene-5-hydroxytetracycline (**20**).[2] But surprisingly this substance could not be induced to cyclize to the corresponding 12-azatetracycline substance (**24**).

We suppose that this is probably due to a high tendency for aromatization of ring C by isomerization of the exocyclic double bond to an endocyclic double bond, giving **23**[2] (the enolization of the 11-carbonyl group can provide the driving force), and a slightly less direct approach of the dimethylamino group to the C(11a) caused by a small steric deformation of ring C due to the now trigonal C(6). The contrast between the easy cyclization of lactone **17** and the failure of lactone **21** to cyclize presents an interesting example of how radically a vicinal group can affect the reactivity of other groups.

Cyclazo-5-oxytetracycline **7**, cyclazo-5-oxytetracycline-nitrile **9**, and cyclazo-α-6-deoxy-5-oxytetracycline **19** are the first derivatives of a new ring system, the 2a,5,5a,6,6a,7,12,12a,13,13a-decahydro-2*H*-benzo[*b*]furo-[2,3,4-*fg*]acridine, shown with its own numbering in **25**.



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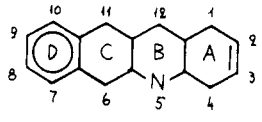

26

However, it is interesting to observe that its similarity to the tetracycline ring system (**1**) allows it to be considered as a 12-azatetracycline (**26**).

Although it was shown that **7** represents the structure of COT hydrochloride, a stereochemical characteristic of it deserves a special comment. The configuration of the former C(11a) of the tetracycline ring system (**1**), now C(6a) of the benzo[*b*]furo[2,3,4-*fg*] acridine ring system (**25**), was tentatively assigned as shown in **7**, based on mechanistic assumptions, steric reasons, and some ^{1}H NMR details. But a more rigorous determination is still needed. This consideration also should be extended to COT nitrile (**9**), CDOT (**19**), lactones **17**, and **21**.

The biological tests made up to now indicate that neither COT (**7**) nor CDOT (**9**) exhibits measurable in vitro activity against *Klebsiella pneumoniae*.[12] This is in accordance with known structure–activity relationships in the tetracycline antibiotics.[1a] Among the different reasons that can lead to the loss of the tetracycline-like activity of these cyclazo compounds, the disruption of the 11,12-β-diketo system can be mentioned. Modifications of this group have produced substances with very little or no activity. A quantum theoretical study of the tetracyclines has implicated that these oxygens are intimately involved in leading to the bacteriostatic effects.[13]

Experimental Section

General. Infrared (IR) spectra were recorded on either a Perkin-Elmer 21 or a Beckman Acculab 4 spectrophotometer as KBr pellets. Proton magnetic resonance (^{1}H NMR) spectra were measured either on a Varian Associates T-60 or an A-60 instrument, with chemical shifts given in parts per million (δ) downfield from tetramethylsilane (δ = 0 ppm) as an internal standard. Coupling constants, *J*, are given in hertz, and s, d, dd, and m indicate singlet, doublet, doublet of doublets, and multiplet, respectively. UV spectra were measured on a Pye Unicam 1800 spectrophotometer. Elemental analyses were determined either by Galbraith Laboratories, Inc., Knoxville, Tenn., or by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.

4-(Aminocarbonyl)-2a,5,5a,6,6a,7,12,12a,12b,12c-decahydro-2a,3,8,12-tetrahydroxy-6,6,12-trimethyl-2,5,7-trioxo-2*H*-benzo[*b*]furo[2,3,4-*fg*]acridinium Chloride (7). (a) A 15-g amount of 11a-chloro hemiketal **2a** was suspended in 50 mL of dry pyridine. Warming was applied with constant stirring. The solid dissolved, and at 90–95 °C yellow crystals precipitated from the brown-green mother liquor. Warming was continued for 1 min more, and the suspension was cooled and filtered. The crystals were washed with ethanol and dried to give 4.5 g (30%) of COT hydrochloride.

The substance was recrystallized by dissolving it in water, adding aqueous NaOH, increasing the pH until 7.0 under nitrogen, filtering, and crystallizing by the addition of aqueous hydrochloric acid up to pH 1.5 as quickly as possible. It was obtained as small yellow prismatic needles: UV (MeOH–HCl, 0.01 N) see Table I; UV (MeOH–NaOH, 0.01 N) λ_{max} (ε) 217 nm (17 200), 249 (18 800), 282 (16 200), 389 (2570); IR 5.62 μm; ^{1}H NMR (the numbering of positions corresponds to that shown in **25**) (TFA) δ 2.09 (3 H, s, CMe), 3.03 (1 H, dd, *J* = 7, 12.5 Hz, H_{12a}), 3.78 (3.5 H, s, NMe), 4.21 (3 H, s, NMe), 4.75 (1 H, d, *J* = 13 Hz, H_{5a}), 4.89 (1 H, d, *J* = 12.5 Hz, H_{6a}), 5.97 (1 H, dd, *J* = 4.5, 7 Hz, H_{12b}); the signal of H_{12c} is probably a doublet of doublets, centered approximately at δ 3.92, of which only one doublet was visible at δ 4.03 (*J* = 4.5 Hz), and the other doublet was covered by the δ 3.78 (NMe) peak; ^{1}H NMR (py-d_5) see Table II.

Anal. Calcd for $C_{22}H_{23}N_2O_9Cl$: C, 53.39; H, 4.68; N, 5.66; Cl, 7.17. Found: C, 53.20; H, 5.10; N, 5.82; Cl, 7.15.

The COT hydrochloride was not very soluble in the usual pure solvents. One of the best solvents found for it was an acetic acid–water (1:1) mixture. A diluted methanolic solution of COT hydrochloride reacted with 0.1 N methanolic silver nitrate solutions, precipitating AgCl, while the starting 11a-chloro hemiketal **2a** did not react in this way.

The amphoteric COT was prepared suspending COT hydrochloride in water and adjusting the pH to 4.0 with aqueous NaOH solution with stirring. The solid was filtered, washed with water until a negative chloride reaction was observed in the washings, and dried.

Anal. Found: Cl, 0.3%.

(b) When in the preparation procedure described above in (a) other solvents such as nitromethane, acetic acid, or ethanol–benzyl chloride (2:1) were substituted for the pyridine, the same COT hydrochloride was obtained. In all of the cases, the IR spectra were identical.

(c) From 11a-Bromo Hemiketal 2c.[7] A 1-g amount of this substance was subjected to the same procedure described above in (a). The obtained crude product was dissolved in water by the addition of aqueous NaOH solution up to pH 7.0 and reprecipitated by the addition of HCl to pH 1.5. The filtrated solid was washed with 1 N aqueous HCl and dried to give 200 mg (22%) of COT hydrochloride. Its IR spectrum was identical with that of the product obtained from 11a-chloro hemiketal **2a**.

4-(Aminocarbonyl)-2a,5,5a,6,12b,12c-hexahydro-2a,3,7,8-tetrahydroxy-6,6,12-trimethyl-2,5-dioxo-2*H*-benzo[*b*]-furo[2,3,4-*fg*]acridinium Chloride (8). A 50-g amount of COT (**7**) as the hydrochloride was added during 2 min to a mixture of 187 g of sulfuric acid and 75 mL of water held at 42–43 °C with constant stirring. Usually the dissolution was complete 2 min after the last addition. The stirring was continued 2 min more, and then the reaction mixture was quenched in 1 L of a water–ice (1:1) mixture, with stirring. The precipitated crude ACOT sulfate was filtered and washed with cold water.

The wet ACOT sulfate was converted into the hydrochloride by suspending it in 150 mL of 2 N aqueous HCl at room temperature, stirring for 1 h, filtering, and washing with water. The crude ACOT hydrochloride was purified by repulping it in 65 mL of cold methanol during 1 h, filtering, washing with cold methanol, and vacuum drying to give 7 g (15%) of greyish-white crystals.

The product was recrystallized by dissolving it in methanol and precipitating it by addition of 1 N aqueous HCl: UV (MeOH–HCl, 0.01 N) see Table I; IR 5.62 μm (aliphatic saturated lactone); ^{1}H NMR (the numbering of positions corresponds to that shown in **25**) (TFA) δ 2.78 (3 H, s, CMe), 3.96 (1 H, dd, *J* = 3.5, 14 Hz, H_{12c}), 4.20 (3 H, s, NMe), 4.60 (3 H, s, NMe), 5.07 (1 H, d, *J* = 14 Hz, H_{5a}), 6.50 (1 H, d, *J* = 3.5 Hz, H_{12b}), 7.17–7.95 (3 H, m, aromatic hydrogens); ^{1}H NMR (py-d_5) δ 2.44 (3 H, s, CCH_3), 4.28 (s, NMe), 4.73 (3 H, s, NMe), 5.48 (1 H, d, *J* = 13.5 Hz, H_{5a}), 6.42 (1 H, d, *J* = 3.5 Hz, H_{12b}); the signal of H_{12c} was probably a doublet of doublets, centered approximately at δ 4.21, of which only one doublet was visible at δ 4.10 (*J* = 3.5 Hz), and the other doublet was covered by the δ 4.28 ($N-CH_3$) peak.

Anal. Calcd for $C_{22}H_{21}N_2O_8Cl$: C, 55.40; H, 4.44; N, 5.88; Cl, 7.44. Found: C, 54.94; H, 4.46; N, 5.80; Cl, 7.30.

4-Cyano-2a,5,5a,6,6a,7,12,12a,12b,12c-decahydro-2a,3,8,12-tetrahydroxy-6,6,12-trimethyl-2,5,7-trioxo-2*H*-benzo[*b*]-furo[2,3,4-*fg*]acridinium Hydroxide Inner Salt (9). To a suspension of 6 g of P_2O_5 in 50 mL of dimethylformamide was added 5 g of COT hydrochloride (7). The mixture was heated with stirring for 5 min in a water bath at 55 °C and cooled in an ice bath; 20 mL of water was added, and the mixture was stirred for 1 h. The solid was filtered, washed with DMF–H_2O (5:2), and dried to give 3 g of crude **9** as the DMF solvate.

On recrystallization, the nitrile formed solvates very tenaciously. The solvents were not eliminated even by prolonged heating at 100 °C under vacuum.

The crude COT nitrile was recrystallized by dissolving it in dimethyl sulfoxide and precipitating it with acetone–water. An acetone solvate was obtained as small white needles. Another procedure was to dissolve it in warm DMF and precipitate with water. Here a DMF solvate was obtained.

A solvent-free product was obtained from water, dissolving with alkali and precipitating with acid, as white long prisms, but frequently slight decomposition occurred: UV (MeOH–HCl, 0.01 N) λ_{max} (ϵ) 233 nm (10 400), 280 (21 000), 352 (3900); ^1H NMR (Me_2SO-d_6) δ 1.77 (s, CMe), 3.41 (s, NMe), 3.76 (s, NMe); IR 4.56 (CN), 5.62 (aliphatic saturated lactone) μm; the presence of DMF was detected by a band at 6.05 μm, and simultaneously the aliphatic saturated lactone band was shifted to 5.60 μm; acetone was detected by a band at 5.83 μm, and again the aliphatic saturated lactone band was shifted to 5.60 μm.

The acetone solvate was analyzed.

Anal. Calcd for $C_{22}H_{20}N_2O_8 0.5C_3H_6O \cdot 1.5H_2O$: C, 56.85; H, 5.28; N, 5.64. Found: C, 57.03; H, 4.85; N, 5.65.

1-[3-Chloro-1,2,3,4-tetrahydro-5-hydroxy-1-methyl-4-oxo-2-naphthyl]-7-(dimethylamino)-1,3,3a,4,7,7a-hexahydro-3a,6-dihydroxy-3,4-dioxoisobenzofuran-5-carboxamide (17). A 50-g amount of α-6-deoxy-5-oxytetracycline (**15**) hydrochloride was dissolved in a mixture of 230 mL of acetone and 50 mL water. With cooling, 16.0 mL of triethylamine, followed by 15.0 g of *N*-chlorosuccinimide, was added. After allowing a 10-min reaction time, 940 mL of water was slowly added with stirring, adjusting the pH to 7.0 with ammonia. After 1 h of agitation, the pH was adjusted to 3.5 with hydrochloric acid and the stirring was continued for 1 additional hour. The mixture was filtered. The obtained solid, which consisted of creamy yellowish crystals containing significant amounts of amorphous material, was purified by dissolving it in ethyl cellosolve, precipitating the crystalline lactone with water, and drying to give 19 g (38%) of lactone **17**. The lactone was recrystallized as white-yellowish short prisms from ethyl cellosolve–water by the same procedure.[14] For analysis, the hydrochloride was prepared from methanol and aqueous HCl as white prisms. The vacuum drying of the lactone was done at room temperature to avoid the risk of cyclization to CDOT (**19**): UV (MeOH–HCl, 0.01 N) see Table I; IR 5.60 μm; ^1H NMR see Table II.

Anal. Calcd for $C_{22}H_{24}N_2O_8Cl_2$: C, 51.27; H, 4.69; N, 5.44; Cl, 13.76. Found: C, 51.19; H, 4.50; N, 5.18; Cl, 13.63.

4-(Aminocarbonyl)-2a,5,5a,6,6a,7,12,12a,12b,12c-decahydro-2a,3,8-trihydroxy-6,6,12-trimethyl-2,5,7-trioxo-2*H*-benzo[*b*]furo[2,3,4-*fg*]acridinium Chloride (19). (a) From Lactone 17. A 1-g amount of lactone **17** was suspended in 10 mL of acetic acid at room temperature. Warming was applied with constant stirring. Soon after dissolution of the lactone, at approximately 80–85 °C, a yellow crystalline precipitate appeared. Stirring was continued for a few minutes more, and the suspension was cooled and filtered. After washing with ethanol, the crystals were dried to give 790 mg (79%) of CDOT hydrochloride. The product was recrystallized in the same way as described for COT (**7**). Yellow rectangular prisms were obtained: UV (MeOH–HCl, 0.01 N) see Table I; IR 5.60 μm; ^1H NMR see Table II.

Anal. Calcd for $C_{22}H_{23}N_2O_8Cl$: C, 55.18; H, 4.84; N, 5.85; Cl, 7.40. Found: C, 54.98; H, 4.94; N, 5.65; Cl, 7.22.

(b) From Lactone 17. When in the preparation procedure described above in a other solvents such as pyridine, nitromethane, or 2-propanol, etc., were substituted for the acetic acid, or if lactone **17** was used as the hydrochloride, the same DCOT was obtained.

(c) From 11a-Chloro-α-6-deoxy-5-hydroxytetracycline (16).[14] A 200-mg amount of 11a-chloro-α-6-deoxy-5-hydroxytetracycline (**16**) was suspended in 0.6 mL of dry pyridine, and warming was applied with stirring. The solid dissolved, and after a few seconds yellow crystals precipitated from the dark mother liquor. The suspension was cooled and filtered. The crystals were washed with ethanol and dried to yield 21 mg (21%) of yellow crystalline DCOT hydrochloride. After recrystallization, it was identical with that obtained in (a) above.

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Registry No.—**1a**, 60-54-8; **1b**, 79-57-2; **2a**, 35689-72-6; **2c**, 39180-53-5; **7**, 68344-82-1; **7** free base, 68421-51-2; **8**, 68344-81-0; **8** sulfate, 68398-33-4; **9**, 68344-79-6; **13**, 7224-60-4; **15**, 564-25-0; **16**, 68344-78-5; **17**, 68344-84-3; **17** free base, 68398-34-5; **19**, 68344-83-2; **21**, 68344-80-9.

References and Notes

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