ml) was stirred and heated under reflux under N_2 for 36 hr. The mixt was filtered and the filtrate was extd with 2 *N* KOH (3 × 10 ml). The combined exts were washed with Et_2O (10 ml), filtered, and cooled, and the product was obtained by careful acidification to pH 1–2, extn with Et_2O, and crystn from EtOAc–hexane, mp 87–91° (lit.[32] mp 90°).

6-Hydroxy-3,4-dihydro-2*H*-1,5-benzodioxepin (84). 1,3-Dibromopropane (200 g) was added to a stirred soln of pyrogallol (62 g) and KOH (55 g) in abs EtOH (330 ml) at room temp. When the initial exothermic reaction had subsided, the mixt was heated under reflux under N_2 for 16 hr, cooled, and filtered and the filtrate was evapd to dryness. The crude product was obtd by extn with Et_2O and distn, bp 128–140° (1 mm). The distillate was stirred with PhH (300 ml) and filtered, the filtrate was evapd to dryness, and the product was obtained by crystn of the residue from petr ether (bp 60–80°), mp 109–110°. *Anal.* ($C_9H_{10}O_3$) C, H.

7-Hydroxy-2,3,4,5-tetrahydro-1,6-benzodioxocin (85) was prepd the same way as the benzodioxepin from pyrogallol and 1,4-dibromobutane; crude product, bp 138–142° (0.8 mm), mp 64–65° from petr ether (bp 60–80°). *Anal.* ($C_{10}H_{12}O_3$) C, H.

8-Hydroxy-4-oxochroman (86). 8-Methoxy-4-oxochroman[33] (1.0 g) was dissolved in dry xylene (10 ml) and anhyd $AlCl_3$ (1.5 g) was added. The mixt was heated at 100° for 1 hr, then decompd with ice-cold 2 *N* HCl and the product was obtained by extn with $CHCl_3$ and crystn from a mixt of PhH and petr ether (bp 60–80°), mp 166–167°. *Anal.* ($C_9H_8O_3$) C, H.

4-Hydroxyxanthene (87). Na (5.0 g) was added in small pieces over 1 hr to a refluxing soln of 4-methoxy-9-oxoxanthene[16] (2.26 g) in *n*-BuOH (80 ml). The mixt was refluxed for 1 hr, the solvent was removed under reduced pressure, and Et_2O extn gave 4-methoxyxanthen, mp 60–61° (crystd from *i*-PrOH). *Anal.* ($C_{14}H_{12}O_2$) C, H. A soln of this product (1.0 g) in dry xylene (25 ml) was treated with anhyd $AlCl_3$ (1.5 g) and the mixt was heated at 100° for 1 hr. After cooling and decompn with ice and 2 *N* HCl, the xylene was removed by steam distn and the product was obtained by filtration and crystn from aq EtOH, mp 121–122°. *Anal.* ($C_{13}H_{10}O_2$) C, H.

1-*N*-Benzylisopropylamino-3-(1,4-dihydro-4-oxo-1-quinolyl)-propan-2-ol (83). 4-Hydroxyquinoline (2.9 g) and the chlorohydrin **81**[6] (4.8 g) were dissolved in EtOH (30 ml) contg NaOEt (2.72 g) and the soln was heated in a sealed tube at 100° for 10 hr. The EtOH was removed under reduced pressure and the product was obtained by extn with $CHCl_3$ and crystn from EtOAc; mp 126–127°, ir ν_{max} (C=O) 1630 cm^{-1}. *Anal.* ($C_{22}H_{26}N_2O_2$) C, H, N.

Anhydro-(3-*N*-benzylisopropylamino-2-hydroxypropyl)-3-hydroxypyridinium Hydroxide (82). 3-Hydroxypyridine (0.95 g) and **81** (2.3 g) were heated on the steam bath for 3 hr. The cooled mixt was dissolved in 2 *N* HCl (30 ml) and the acidic soln was washed with $CHCl_3$ (2 × 20 ml) and basified, and the product was obtained by extn with $CHCl_3$ and crystn from EtOAc; mp 142–143°, uv λ_{max} (H_2O) 217 mμ (ε 25,620), 253 (7110), 327 (5430); (1 *N* NaOH) 252 (7320), 325 (5040); (1 *N* HCl) 231 (4830), 292 (4380). *Anal.* ($C_{18}H_{24}N_2O_2$) C, H, N. 3-Hydroxypyridine methochloride[34] had λ_{max} (H_2O) 213 mμ (24,640), 249 (8120), 320 (57,810); (0.1 *N* NaOH) 245 (9000), 322 (5100); (0.1 *N* HCl) 288 (5860). The same product was obtained in the presence of NaOEt.

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Synthesis and Biological Activity of Acronycine Analogs

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The synthesis and biological activity of compounds related to acronycine (**3a**) are reported. None of the derivatives and analogs prepared showed enhanced activity against experimental tumors in mice and rats over the parent alkaloid.

Acronycine (**3a**), an acridone alkaloid, has been reported to have broad spectrum antitumor activity in experimental animals.[1-3] The structure of acronycine was confirmed by numerous workers[4-6,†] and by the synthetic work

†J. Z. Gougoutas and B. A. Kaski, personal communication to J. R. Beck cited in ref 7.

of Beck, *et al.*[7] Recently the synthesis carried out by Hlubucek[8] made noracronycine (1) and *N*-desmethylnoracronycine (**6a**) readily available. While attempts to improve the activity of acronycine by structural modifications have been reported,[2] this work was restricted only to the pyran part of the molecule.

Since noracronycine was reported to be inactive[2] and differs from acronycine only by replacement of OMe by OH, we undertook the synthesis and biological evaluation of a variety of 6-alkoxy derivatives of noracronycine (**3b–f**, Scheme I). Also prepared and evaluated were the 6-AcO (**3g**), the N,O-Et$_2$ (**9**), and the 9-Cl (**8**) analogs (Scheme II). All of these compounds were prepared by standard techniques.

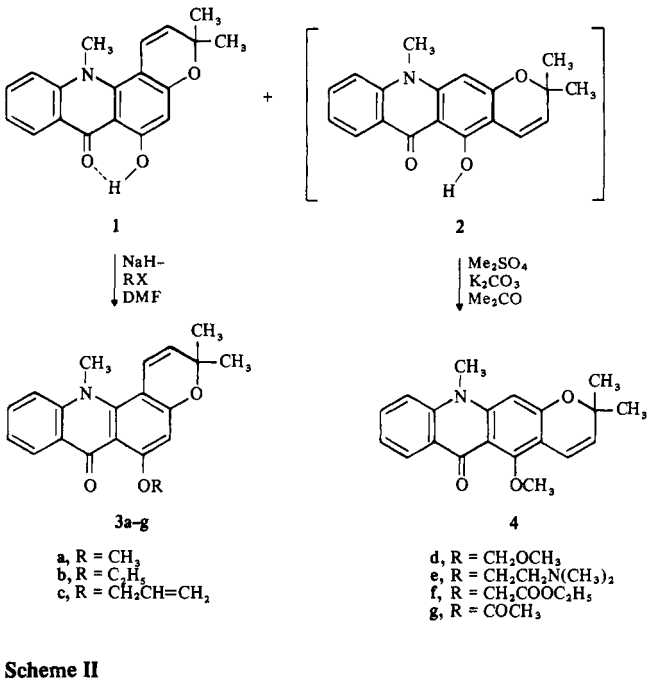
Ethylation of *N*-desmethylnoracronycine (**6a**) gave mainly **9** and a small amount of **10**. 9-Chloroacronycine (**8**) and 9-chlorononoracronycine (**7**) were obtained by methylation of **6b**, which in turn, was prepared from crude **5b**, in 14% yield by the same procedures used for the preparation of **5a**.[7]

Hlubucek, *et al.*, reported that in their synthesis of noracronycine (**1**) no linear analog of **1**, *i.e.*, **2** could be detected by tlc.[8] However, by methylation of the mother liquors obtained from **1** in the reported synthesis, we were able to isolate a small quantity of isoacronycine (**4**).

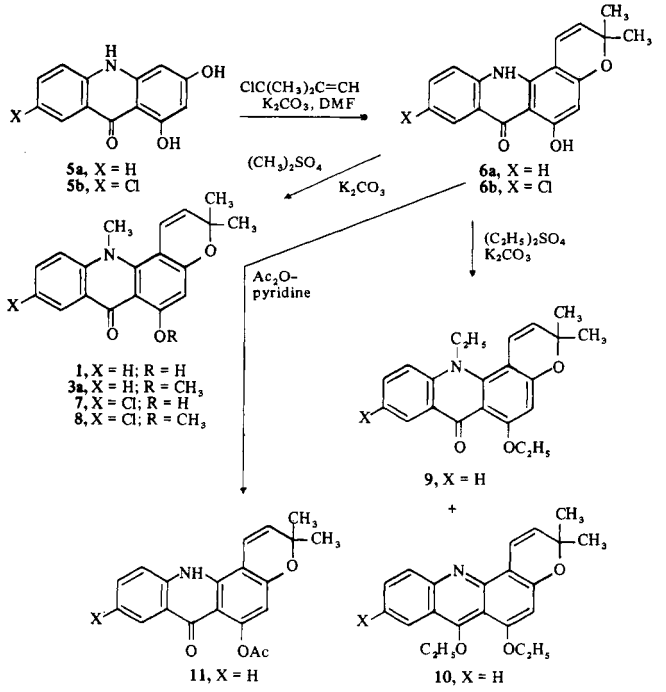
The synthesis of acronycine analogs was then extended to molecules based on the xanthone rather than the acridone nucleus (Scheme III). Condensation of 1,3-dihydroxyxanthone (**12**) and 2-chloro-2-methylbutyne in a basic medium at 60° afforded the acetylenic ether (**13**). Repetition of this procedure at 65° led to a mixture of both **13** and the ring-closed compounds **14** and **15**. In boiling PhNEt$_2$, the rearrangement of **13** was completed and pure **15** and an inseparable mixture of **14** and **15** were isolated. However, upon methylation, the corresponding Me ethers **16** and **17**, respectively, were easily separated by column chromatography. The assigned structures are based on the reductive detosylation of **18** (derived from **15**) to **19**.‡ The nmr spectrum is in agreement with the proposed structure, *i.e.*, 2 singlets for para-aromatic protons. Since under the same conditions, **14** would not be expected to give an nmr spectrum compatible for para-aromatic protons, structure **16** was assigned as the acronycine analog derived from the cyclization mixture.

Biological Results.§ **(1) Antitumor Activity.** Sixteen compounds (**1**, **3a–g**, **4**, **6a**, **8**, **9**, **11**, **15**, **16**, **17**) were tested for antitumor activity against the solid tumor form of sarcoma 180 and, with the exception of **3d** and **3e**, against the

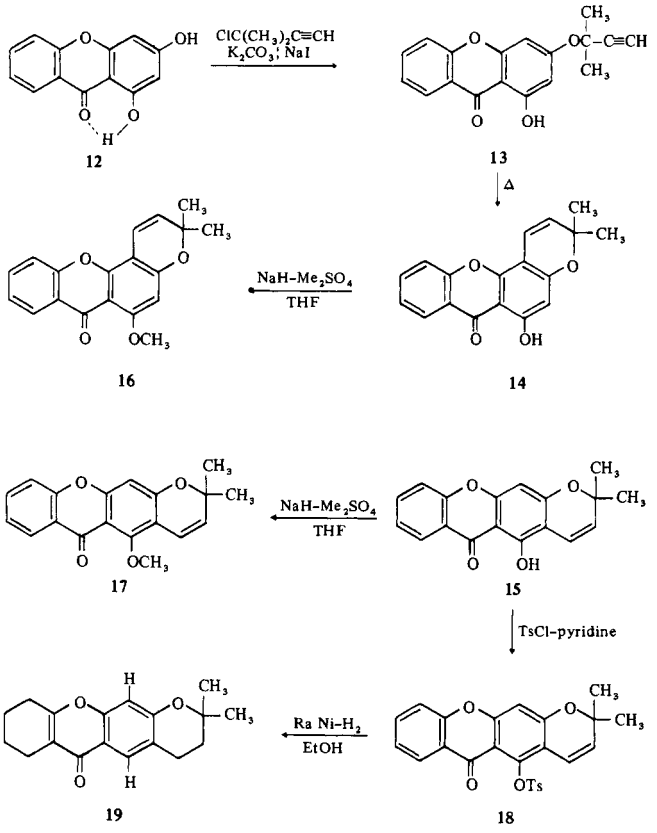
Scheme I



Scheme II



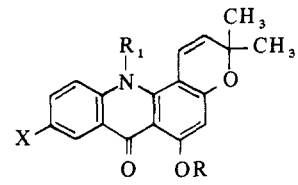
Scheme III



‡A similar reductive detosylation was carried out by T. R. Gorindachari and coworkers on tosylnoracronycine, *cf.* ref 5.

§For *in vitro* and *in vivo* test methodologies, see ref 9.

Table I. Acronycine Analogs



No.	Method of prepn	Reactant	R	R_1	X	Yield, %	Recrystn solvent	Mp, °C	Appearance	Formula[d]
3b	A	Et_2SO_4	Et	Me	H	58	CH_2Cl_2-hexane	163-164.5	Light yellow prisms	$C_{21}H_{21}NO_3$
3c	A	CH_2=$CHCH_2Br$	CH_2=$CHCH_2$	Me	H	32	Et_2O	122-124	Fine light yellow needles	$C_{22}H_{21}NO_3$
3d	A	CH_3OCH_2Cl	CH_3OCH_2	Me	H	50.7	EtOAc-Et_2O	137-139.5	Light yellow prisms	$C_{21}H_{21}NO_3$
3e	A	$(CH_3)_2NCH_2CH_2Cl$	$(CH_3)_2NCH_2CH_2$	Me	H	74	CH_2Cl_2-hexane	148-150	Yellow prisms	$C_{23}H_{26}N_2O_5$
3f	A[a]	$BrCH_2COOH$	CH_2COOEt	Me	H	30	Et_2O	98-100	Light yellow prisms	$C_{23}H_{23}NO_5$
3g	B	Ac_2O	Ac	Me	H	58	EtOAc	201-203	Yellow rods	$C_{21}H_{19}NO_4$
7	C[b]	Me_2SO_4	H	Me	Cl	14.4	CH_2Cl_2	259.5-261.5	Orange prisms	$C_{19}H_{16}ClNO_3$
8	C[b]	Me_2SO_4	Me	Me	Cl	46.1	EtOAc	223.5-225.5	Yellow rectangular plates	$C_{20}H_{18}ClNO_3$
9	C[a,c]	Et_2SO_4	Et	Et	H	39	EtOAc-Et_2O	139-139.5	Yellow prisms	$C_{22}H_{23}NO_3$
11	B	Ac_2O	Ac	H	H	67	$CHCl_3$-EtOAc	235-236.5	Almost colorless fluffy needles	$C_{20}H_{17}NO_4$

[a] Isolated by chromatography on silica gel with hexane-EtOAc as eluent. [b] Starting material **6b** was obtained as orange prisms, mp 258-262.5°; *Anal.* ($C_{18}H_{14}ClNO_3$) C, H, N. [c] 4.4% of **10** was isolated as by-product. Ir of **10** shows no CO of 1630 cm^{-1}; *Anal.* ($C_{22}H_{23}NO_3$) C, H, N. No compound analogous to 7 (R' = Et, X = H) has been isolated. [d] All compds analyzed correctly for C, H, N.

Ehrlich carcinoma solid tumor in mice. In addition, **3a** (acronycine) was tested against both the solid and ascitic forms of leukemia L1210 and the ascitic form of Ehrlich carcinoma in mice and against the Walker 256 carcinosarcoma in rats. Compd **1** was also tested against the solid form of leukemia L1210 and **3e** and **8** were tested against Ehrlich ascites in mice.

In mice injected with sarcoma 180, ip treatment with acronycine **(3a)** at 200 mg/kg once daily for 8 days caused a 50% reduction in the average tumor weight as compared to untreated controls. Acronycine failed to produce a significant reduction of tumor weights when administered ip to mice bearing the solid form of either Ehrlich carcinoma or leukemia L1210 or, upon oral administration, to prolong the life of mice injected with the ascitic form of leukemia L1210. These results are in line with those reported by Svoboda, *et al.*,[1-3] although, because of differences in methodology, the studies cannot be directly compared. Acronycine was reported by these investigators to have no effect on the Walker carcinosarcoma 256 tumor in rats or on the survival of mice infected with the ascitic form of Ehrlich carcinoma.[3] However, in the present studies, the average weight of the Walker 256 tumors in rats treated with **3a** at a dose of 100 mg/kg per day was one-third that of the tumors in untreated control animals. In addition, a 54% prolongation of life was observed in mice injected with the ascitic form of Ehrlich carcinoma and treated for 8 days with **3a** at 200 mg/kg orally.

Among the derivatives of acronycine which were tested, only **3e** exhibited significant antitumor activity causing a 52% reduction in tumor size in mice bearing the solid form of sarcoma 180 and a 60% prolongation of the lives of mice injected with Ehrlich ascites following oral treatment for 8 days with the MTD of 10 mg/kg per day.

(2) Other Biological Activities. No significant *in vitro* antibacterial activity was observed when **3a** and **15** were tested against representative Gram-positive and Gram-negative bacteria nor when **3a** was tested against the yeast, *Candida albicans,* and the dermatophytes, *Trichophyton mentagrophytes* and *Microsporum audouini.*

Various compds were tested for their antiviral, antibacterial, antiprotozoal, and anthelmintic activity *in vivo.* Although acronycine itself was without effect, a number of its derivatives were found to exert an effect against the local *Trichomonas vaginalis* infection of mice following infiltration of the substances into the site of infection. However, the 3 most active compds, **4, 3d,** and **3g,** failed to show any activity against the *T. vaginalis* local infection upon oral administration. None of the compds tested exhibited any activity against Columbia SK and influenza A (PR8) virus or representative Gram-positive and Gram-negative bacterial systemic infections in mice nor against the local *Candida albicans* and the natural pinworm *(Syphacia obvelata)* infections in mice. Compds **3a, 15,** and **17** were also without effect when administered orally to rats infected intracecally with *Endoamoeba histolytica.*

Experimental Section#

Method A. 3,12-Dihydro-6-ethoxy-3,3,12-trimethyl-7*H*-pyrano[2,3-*c*]acridin-7-one (3b). A soln of 6.14 g (20 mmoles) of **1** in 70 ml of anhyd THF together with 2.4 g (60 mmoles) of a 60% NaH dispersion in oil was stirred and refluxed under N_2 for 2 hr when an excess of Et_2SO_4 was added and the reaction mixt was heated and stirred until the reaction had gone to completion (tlc estimation). After standing overnight at room temp, the mixt was dild with ice and H_2O. A yellow solid (5.8 g) was collected and twice recryst from CH_2Cl_2-hexane; 3.9 g (58%) of **3b** was obtained as light yellow prisms, mp 163-164.5°.

Method B. 3,12-Dihydro-6-acetoxy-3,3,12-trimethyl-7*H*-pyrano[2,3-*c*]acridin-7-one (3g). A soln of 5.0 g (16.3 mmoles) of **1** and 5 g of Ac_2O in 100 ml of pyridine was stirred at 85°. After 1 day,

#Melting points were detd with a Thomas-Hoover capillary melting point app and are corrected. Elemental analyses were within ±0.4% of the theoretical values. Nmr spectra were obtd on all compounds with a Varian Associates Model A-60 or HA-100 spectrometer. Ir spectra were measured on a Beckman IR-9 instrument.

another 5 g of Ac_2O was added and the mixt was continuously heated and stirred for another day. The solvent was evaporated. Last traces of pyridine were removed by repeated evaporation with PhMe. The residue was taken up in hot EtOAc, filtered, and concentrated. On cooling, 3.87 g of almost pure material (mp 201–203°) was obtained which was recrystallized from EtOAc to give 3.3 g (58%) of yellow rods, mp 201–203°, lit.[10] mp 160–166.5°.

Method C. 9-Chloro-3,12-dihydro-6-hydroxy-3,3,12-trimethyl-7*H*-pyrano[2,3-*c*]acridin-7-one (7) and 9-Chloro-3,12-dihydro-6-methoxy-3,3,12-trimethyl-7*H*-pyrano[2,3-*c*]acridin-7-one (8). A mixt of 12.0 g (36.6 mmoles) of **6b** in 400 ml of anhyd Me_2CO, 138 g of powdered anhyd K_2CO_3, and 12.6 g of Me_2SO_4 was stirred in a flask fitted with an overhead stirrer. After 18 hr, another 8 g of Me_2SO_4 and, again 7 hr later, 8 g of Me_2SO_4 along with 25 g of anhyd K_2CO_3 were added. After a total of 40 hr reaction time, the solvent was evapd and 250 ml of H_2O was added. A crystalline solid was collected and washed with H_2O. The dry material was slurried in hot EtOAc. The EtOAc filtrate was concentrated and the resulting ppt combined with the remaining solid to a total of 9.8 g. This material was chromatographed on 360 g of silica gel with CH_2Cl_2 as eluent. From the first fractions 1.8 g (14.4%) of orange prisms (**7**), mp 259.5–261.5°, was obtained. The main product was eluted with EtOAc. Concentration of the eluate afforded 6.0 g (46.1%) of bright yellow, rectangular plates (**8**) (mp 223.5–225.5°) and 0.75 g (mp 216.5–219.5°) as a second crop. *Anal.* (**7**) ($C_{19}H_{16}ClNO_3$) C, H, N. *Anal.* (**8**) ($C_{20}H_{18}ClNO_3$) C, H, N.

2,11-Dihydro-5-methoxy-2,2,11-trimethyl-2*H*,6*H*-pyrano[2,3-*c*]-acridin-6-one (4) (Isoacronycine). Crude **1** (2 g) (obtained by the concentration of the mother liquors from the preparation of **1**[8]) and 1 g of a 60% dispersion of NaH in oil were refluxed in anhyd THF for 2 hr under N_2. A large excess of Me_2SO_4 was added. The mixt was allowed to stir for approximately 30 min. Solvent was evapd, H_2O was added, and the mixt was warmed on the steam bath to destroy excess Me_2SO_4. After cooling, a solid was collected which was taken up in CH_2Cl_2, dried (Na_2SO_4), and evapd to dryness. From the obtained solid, isoacronycine **4** was eluted by column chromatography on silica gel with hexane–EtOAc, 1:1 as eluent. Recrystallization from cyclohexane yielded 50 mg of bright yellow prisms, mp 163–165° (lit.[11] 162.5–164° for **4** *via* an independent synthesis; *Anal.* ($C_{20}H_{19}NO_3$) C, H, N.

1-Hydroxy-3-(2-methyl-3-butyne-2-oxy)xanthone (13). A mixt of 5.02 (22 mmoles) of **12**, 14 g of powdered anhyd K_2CO_3, 0.75 g of NaI, and 5.125 g (50 mmoles) of 2-chloro-2-methylbutyne in 50 ml of DMF was stirred under N_2 for 18 hr at 60° (oil bath). An additional 5.125 g of 2-chloro-2-methylbutyne was added after 24 hr. Two days later, the reaction mixt was partitioned between 100 ml of H_2O, 50 ml of 2 *N* aq NaOH, and 250 ml of EtOAc. The aqueous layer was again twice extd with 250-ml portions of EtOAc. The combined organic layers were washed twice with 200 ml of H_2O and once with saturated brine, then dried (Na_2SO_4), and evapd to dryness. On treatment with Et_2O and petr ether (40–60°) a crude material of 174–176° was obtained. It was dissolved in $CHCl_3$ and filtered thoroughly through 10 g of Florisil. Concentration of the eluate and crystallization from $CHCl_3$, Et_2O, and petr ether yielded 1.65 g (25.5%) of colorless prisms, of mp 177–178°. Recrystallization from Et_2O afforded an analytical sample, mp 179–180°. *Anal.* ($C_{18}H_{14}O_4$) C, H, N.

5-Hydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (15). A mixt of 68.5 g (300 mmoles) of **12** in 400 ml of dry DMF, 165.6 g of powdered anhyd K_2CO_3, and 4.5 g of NaI was stirred at 65° (oil bath) with an overhead stirrer in a 3-necked, 2-l. flask fitted with a condenser with drying tube and N_2 inlet and a dropping funnel. The system was filled with N_2. Over a period of 2 hr, 51.25 g (500 mmoles) of 2-chloro-2-methylbutyne was added. After 24 hr, another 20.5 g (200 mmoles) of 2-chloro-2-methylbutyne was added. After a total of 3 days, the reaction mixt was poured into 500 ml of H_2O and extracted twice with 500- and 250-ml portions of H_2O, dried (Na_2SO_4), filtered over 400 g of Florisil, and washed thoroughly with C_6H_6. Concentration of this soln to 300 ml, addition of hexane, and cooling overnight gave 40.9 g (46.4%) of light yellow prisms, mp 140–160°. All attempts to raise the mp by recrystallization in various solvents failed.

A soln of 37.05 g (126 mmoles) of this mixt in 750 ml of freshly distilled $PhNEt_2$ was heated for 1 hr under N_2 to gentle reflux. The solvent was evaporated. The residue was taken up in 200 ml of $CHCl_3$ and 200 ml of Et_2O was added. After cooling overnight, 24.0 g (64.8%) of light yellow fine needles (**15**), mp 171–172°, was collected. Recrystallization from $CHCl_3$–Et_2O raised the mp to 171.5–172.5°.

The filtrate was concentrated. When adding Et_2O and petr ether (40–60°), two crops (6.7 and 1.3 g of mp 135–150° and 130–160°) were obtained. Recrystallization from CH_2Cl_2–Et_2O gave 7.0 g (18.8%) of a 2:1 mixt of **14** and **15** by nmr. *Anal.* ($C_{18}H_{14}O_4$) C, H, N.

6-Methoxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one (16) and 5-Methoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (17). To a stirred soln of 6.47 g (22 mmoles) of the above 2:1 mixt of **14** and **15** in 100 ml of abs THF was added 2.0 g (50 mmoles) of a 60% NaH dispersion in oil previously rinsed with petr ether (40–60°). After refluxing for 1 hr, 6.3 g of Me_2SO_4 in 30 ml of THF was added over a period of 30 min. When tlc (hexane–EtOAc, 1:1) indicated the disappearance of the starting material, the excess NaH was destroyed with MeOH. A formed ppt was filtered off. The filtrate was evapd to dryness and the residue chromatographed on 500 g of silica gel with EtOAc–hexane (1:1) as eluent. Fractions of the same kind were combined and concentrated *in vacuo*. The residues were crystallized from EtOAc and Et_2O and afforded 1.2 g (17.7%) of **17** as light yellow, almost colorless rods of mp 127.5–128.5° and 2.9 g (42.8%) of **16** as light yellow, almost colorless plates, mp 197–198°. *Anal.* (**17**) ($C_{19}H_{16}O_4$) C, H, N. *Anal.* (**16**) ($C_{19}H_{16}O_4$) C, H, N.

5-Methoxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (17). To a stirred soln of 14.82 g (50 mmoles) of **15** in 300 ml of anhyd THF was added 4.0 g (100 mmoles) of a 60% NaH dispersion in oil previously rinsed with petr ether (40–60°). After refluxing for 1 hr, 12.6 g of Me_2SO_4 was added dropwise within 1 hr. Then another 2 g of NaH dispersion in oil (purified as described above) was added and after 30 min, again 6.3 g of Me_2SO_4 was dropped into the soln. The mixt turned soon colorless and was allowed to stand overnight at room temp. Excess NaH was destroyed with MeOH and the formed ppt was filtered. The filtrate was concentrated, taken up with EtOAc, and filtered thoroughly through 400 g of Florisil. This soln was concd. Addition of Et_2O afforded 8.5 g (mp 125–127°), and as second crop 2.0 g (mp 123.5–127°), a total of 68.1% of very light yellow rods. Recrystallization from EtOAc, Et_2O yielded pure material of mp 126.5–127.5° identical by mmp with **17** separated in the previously described reaction.

5-*p*-Toluenesulfonyloxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]-xanthen-6-one (18). A mixt of 0.5 g (1.7 mmoles) of **15**, 1.2 g of TsCl, and 4 g of powdered anhyd K_2CO_3 in 70 ml of Me_2CO was stirred for 6 hr at reflux. After standing overnight at room temp, the mixt was concd to a thick slurry, then diluted with 100 ml of H_2O and allowed to stir for 2 hr. White needles (610 mg, 80%), mp 220–220.5°, were filtered. An analytical sample melted at 221–221.5°. *Anal.* ($C_{25}H_{20}O_6S$) C, H, N.

2,2-Dimethyl-3,4,7,8,9,10-hexahydro-2*H*,6*H*-pyrano[3,2-*b*]-xanthen-6-one (19). To a slurry of 610 mg (1.36 mmoles) of **18** in 80 ml of EtOH was added 12 g of wet Raney Ni catalyst previously washed with EtOH. H_2 was bubbled in a slow rate through the boiling and stirred reaction mixt for 7 hr. After standing for 2 days at room temp, the catalyst was filtered over Celite and washed carefully with EtOH. The filtrate was evapd and the residue separated by thick-layer chromatography (hexane, EtOAc, 1:1). The main product was extracted with $CHCl_3$. The extract was evapd to dryness and the residue crystallized from Et_2O–petr ether (40–60°), 95 mg (24.6%), mp 169.5–171° of colorless prisms were obtained. *Anal.* ($C_{18}H_{20}O_3$) C, H, N.

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Metabolism of 1-[(5-Nitrofurfurylidene)amino]-2-imidazolidinone†

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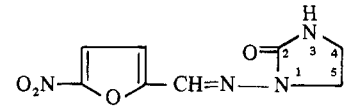
The metabolism of nifuradene (1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone) (I), a urinary tract antibacterial agent, was investigated in the human. Two metabolites, 1-[(5-nitrofurfurylidene)amino]-hydantoin (nitrofurantoin) (II) and 4-hydroxy-1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone (III), were isolated and identified. A degradation product of the labile metabolite III was also isolated and identified as 1-[(5-nitrofurfurylidene)amino]-4-imidazolin-2-one (IV). An oxidative metabolic pathway of I through III to II is proposed.

Early work on the metabolism of nitrofuraldehyde derivatives emphasized reduction of the nitro group. A variety of evidence accumulated indicating the presence of the reduced form,[1] but isolation of the product was not achieved. Then Ebetino, *et al.*,[2] successfully synthesized a series of aminofurans and their acetylated derivatives after chemical reduction of the corresponding nitrofurans; and Olivard, *et al.*,[3] isolated the metabolic reduction product 5-acetamido-2-furaldehyde acetylhydrazone from urine of rabbits fed 5-nitro-2-furaldehyde acetylhydrazone.

In this communication, we present evidence of a primary oxidative metabolic pathway involving the side chain of a nitrofuraldehyde derivative. The compound under investigation was nifuradene, I,[4] a promising urinary tract antibacterial agent.



I

Results

A. Isolation and Identification of Products. Earlier chromatographic work by D. Humphrey of these laboratories had indicated the presence of yellow metabolites of I in rat and dog urine. Their isolation was initiated in the present study, and prep tlc of the nitromethane extract of human urine from subjects administered I indicated the presence of 4 major bands. These bands had average R_f values of 0.96, 0.88, 0.80, and 0.68 in Me_2CO-MeOH (see Experimental Section). However, during preliminary investigations of these compounds, the material with R_f 0.80 would split into 2 bands upon being rechromatographed. The resulting second band had an R_f 0.88, corresponding to 1 of the original bands. Material from these R_f 0.88 bands had identical uv and ir spectra, and behaved identically during chromatography. The material with R_f 0.80 was labile, and was degrading to the material of R_f 0.88. Qualitative experiments indicated that thermolability was the major cause of conversion. Fresh urine samples showed no evidence of the R_f 0.88 material if they were rapidly processed and chromatographed at temps not exceeding 30°.

All 3 excretion products, and the degradation product, were isolated in small quantities in crystalline form. Because of the small quantities available, uv, ir, and nmr spectrometry were relied upon extensively for product identification.

The excretion product of R_f 0.68 in solvent system 1 was unaltered I. The uv, ir, and nmr data for the excretion product all agreed with those for authentic I. Cochromatography of the product and authentic I in 2 paper and 2 tlc systems did not separate the components. The minimal inhibitory concn (MIC) of I against *Escherichia coli* was 0.19 μg/ml, which is equal to the MIC of authentic nifuradene.

The excretion product having R_f 0.96 in solvent system 1 was nitrofurantoin, *i.e.*, 1-[(5-nitrofurfurylidene)amino]-hydantoin (II). In the form of the hydrate, both the product and authentic II yielded identical uv, ir, and nmr data. No separation of components was observed during cochromatography in 2 paper and 2 tlc systems.

The third excretion product of R_f 0.80 in solvent system 1 had ir absorption bands at 1250 and 1015 cm^{-1} indicating that the furan ring remained intact.[5] Bands at 1505 and 1335 cm^{-1} further indicated that NO_2 was still present,[6] and led to the conclusion that metabolic attack on I had been initiated in the imidazolidinone ring portion of the compound. Since both the C=O stretching band (1705 cm^{-1}) and the NH stretching band (3380 cm^{-1})[6] appeared in the spectrum, the reaction appeared to be initiated at the 4 or 5 CH_2 group (see Scheme I).

Further data concerning the structure of this compound, and the other isolated urinary materials, were obtained by nmr spectrometry. The nmr signals obtained with the R_f 0.80 compound are listed in Table I under number III. The doublets at δ 7.01 and 7.71 ppm are attributed to the 2 furan ring protons. Their location agrees well with those of the other nitrofurans listed. The singlet at δ 7.59 must result from the azomethine proton, since no protons are present on adjacent atoms and no splitting of the peak is expected or found. This confirms the ir data on the integrity of the 2-furaldehyde moiety. There are 3 bands (quadruplets; δ 3.50, 3.88, 5.27) representing the imidazolidinone ring in the spectrum of III, whereas only 2 bands (δ 3.50, 3.76) are found in the spectrum of I. These 3 sets of quadruplets are typical of a CH_2CH= group corresponding to an

†United States Adopted Name (USAN): nifuradene.