(40) J. Robak, A. Wieckowski, and R. Gryglewski, *Biochem. Pharmacol.*, **27**, 393 (1978).
(41) G. Levy and C. G. Regardh, *J. Pharm. Sci.*, **60**, 608 (1971).
(42) G. Ramachander, F. D. Williams, and J. F. Emele, *ibid.*, **62**, 1498 (1973).
(43) B. H. Thomas, W. Zeitz, and B. B. Coldwell, *ibid.*, **63**, 1367 (1974).
(44) L. W. Whitehouse, C. J. Paul, and B. H. Thomas, *ibid.*, **64**, 819 (1975).
(45) L. T. Wong, G. Solomonraj, and B. H. Thomas, *Xenobiotica*, **6**, 575 (1976).
(46) L. W. Whitehouse, C. J. Paul, L. T. Wong, and B. H. Thomas, *J. Pharm. Sci.*, **66**, 1399 (1977).
(47) M. J. H. Smith and J. D. Irving, *Br. J. Radiol.*, **28**, 39 (1955).
(48) R. C. Heading, J. Nimmo, L. F. Prescott, and P. Tothill, *Br. J. Pharmacol.*, **47**, 415 (1973).
(49) J. Nimmo, R. C. Heading, P. Tothill, and L. F. Prescott, *Br. Med. J.*, **1**, 587 (1973).
(50) J. A. Clements, R. C. Heading, W. S. Nimmo, and L. F. Prescott, *Clin. Pharmacol. Ther.*, **24**, 420 (1978).
(51) C. P. Siegers, O. Strubelt, and G. Back, *Eur. J. Pharmacol.*, **20**, 181 (1972).
(52) C. P. Siegers, *Pharmacology (Basel)*, **10**, 19 (1973).

ACKNOWLEDGMENTS

The authors thank P. Besamusca, F. Hagelen, H. Heinen, Y. de Jong, A. van de Kuil, and P. W. M. Zeylmans for excellent technical assistance. They also thank Dr. M. Parnham and Mrs. G. Franchimont for their assistance in manuscript preparation.

This study constitutes part of Joint Project No. 110/79 of the National Institute of Public Health with the Health Inspection for Drugs and the State University of Utrecht.

Rearrangement of Chloramphenicol-3-monosuccinate

D. A. BRENT *x, P. CHANDRASURIN *, A. RAGOUZEOS *, B. S. HURLBERT *, and J. T. BURKE ‡

Received July 19, 1979, from the **Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, NC 27709*, and the ‡*School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514.* Accepted for publication March 7, 1980.

Abstract □ The equilibrium mixture of chloramphenicol-3-monosuccinate and its alternate form at neutral pH in aqueous solution was reexamined. The structure of the alternate form was shown by mass spectrometry and NMR spectroscopy to be chloramphenicol-1-monosuccinate and not the cyclic hemi-ortho ester reported previously.

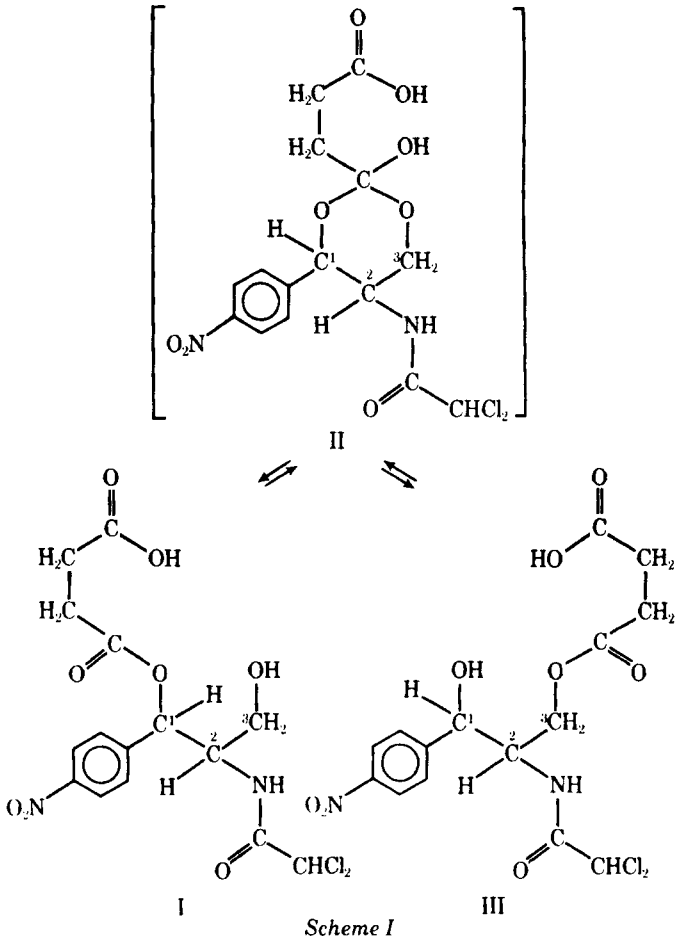
Keyphrases □ Chloramphenicol-3-monosuccinate—identification of alternate form in equilibrium mixture as chloramphenicol-1-monosuccinate □ Antibacterials—chloramphenicol, identification of 1-succinate ester as alternate form in equilibrium mixture with 3-succinate ester □ Prodrugs—chloramphenicol-3-monosuccinate, identification of 1-succinate ester as alternate form in equilibrium mixture with 3-succinate ester

The sodium salt of chloramphenicol-3-monosuccinate[1] (III) is used as a prodrug, generating the broad spectrum antibiotic chloramphenicol by hydrolysis of the succinate ester in the liver (1). Sandmann *et al.* (2) reported that at neutral pH, III exists in equilibrium with a different molecular form, which they identified as a cyclic hemi-ortho ester (II) (Scheme I).

RESULTS AND DISCUSSION

By using a recently developed, sensitive, high-pressure liquid chromatographic (HPLC) assay for III, three products were detected after equilibrating III in aqueous solution at pH 7.5 for 24 hr (3). The first and third peaks (Fig. 1) were identified as chloramphenicol and III, respectively, by comparison with standards and by mass spectrometry of their trimethylsilyl ethers. The mass spectrum of the trimethylsilyl derivative of the first HPLC peak agreed with spectra published for *O*-bis(trimethylsilyl)chloramphenicol (4–6). The mass spectrum of the trimethylsilyl derivative of the third HPLC peak gave ions consistent with IV: *m/e* 551 (2.6%, M − CH_3), 483 (0.6), 377 (2.92), 225 (75.6), 224 (28.9), 173 (25.2), and 73 (100.0).

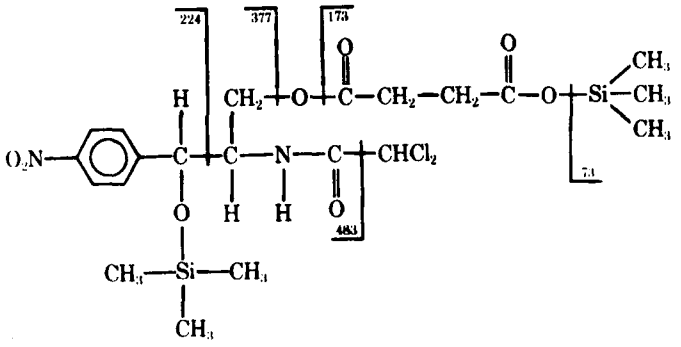
The second eluted compound exhibited an NMR spectrum identical to that reported by Sandmann *et al.* (2) for II. However, the mass spectrum of the trimethylsilyl derivative of this compound supported the structure of chloramphenicol-1-monosuccinate (I) and not the cyclic hemi-ortho ester (II). The ion having the greatest mass was *m/e* 551 (1.7%,



Scheme I

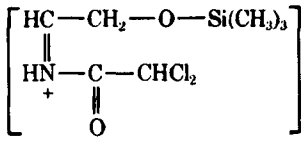
[1] The USAN name chloramphenicol sodium succinate refers to the sodium salt of the 3-monosuccinate ester.

0022-3549/80/0800-0906$01.00/0

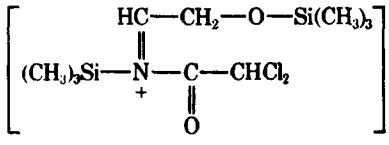


IV (mol. wt. 566)

M − CH_3). The base peak was *m/e* 73. The accurate masses of *m/e* 551 (2.7%), 242 (10.6), and 314 (4.57) were measured by peak matching at 10,000 resolution and 10% valley definition; their accurate masses were 551.0838, 131.0161, and 314.0569, respectively. The corresponding elemental compositions are $C_{20}H_{29}Cl_2N_2O_8Si_2$ (error of 0.1 millimass unit), $C_7H_{14}Cl_2NO_2Si$ (1.0), and $C_{10}H_{22}Cl_2NO_2Si_2$ (0.3). These peaks are consistent with bis- and tris(trimethylsilyl) derivatives of I. The mass spectrum supports the structure of I, but it is unclear whether the derivatizing reagent, which contains a weak base, could cause II to rearrange to I prior to silylation.



m/e 242



m/e 314

The evidence reported for the alternate form of III, having the structure denoted as II, was reexamined to determine if it really excluded the possibility of this product being I. Sandmann *et al.* (2) reported that chemical evidence indicated that the O→O migration product was incapable of existence in equilibrium under experimental conditions, but they did not describe experiments to prove this point. Within the knowledge of the present investigators, I never has been synthesized. The IR spectrum of III shows three distinct carbonyl absorptions (1690, 1718, and 1745 cm^{-1}), but only two absorptions (1690 and 1745 cm^{-1}) were shown for the alternate form (2). Sandmann *et al.* (2) concluded that this result was due to loss of the ester carbonyl. However, the band at 1718 cm^{-1} is likely the acid carbonyl, which has coalesced with one of the two remaining carbonyl bands or has shifted to 1600 cm^{-1} if it is in the form of a carboxylate anion (7). The optical rotatory dispersion curve for III shows a positive Cotton effect, while the curve for the alternate form shows a negative Cotton effect (2). The optical rotatory dispersion curves for O^1-acetylchloramphenicol-3-monosuccinate and O^1-dichloroacetylchloramphenicol-3-monosuccinate show negative Cotton effects

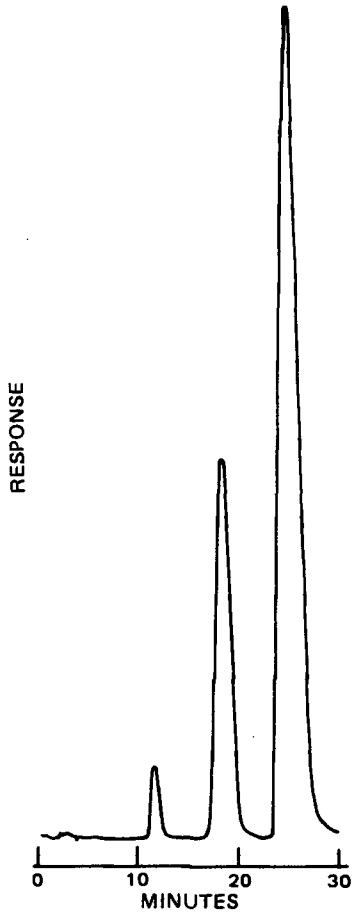


Figure 1—*High-pressure liquid chromatogram of the equilibrium mixture of I. The pH of the mobile phase was 2.5.*

(2). This evidence, used to support Structure II, also supports Structure I.

Sandmann *et al.* (2) reported that attempts to methylate the alternate form of III resulted in complex mixtures. In the present investigation, methylation of the alternate form with diazomethane in ether gave methyl esters of III and I if the reaction was allowed to stand with excess diazomethane. If the diazomethane was added and followed immediately by the addition of acetic acid, only the methyl ester of I resulted. These reactions were followed by HPLC and NMR. The NMR parameters of these products are shown in Table I.

One key factor causing Sandmann *et al.* to assign the alternate form of III as II was the absence of a hydroxylic proton in the NMR spectrum (2). As shown in Table I, this proton is visible in the NMR spectrum of the methyl ester of the alternate form taken in dimethyl sulfoxide-d_6. Furthermore, the downfield shift for H-1 of the alternate form compared with its shift in III is attributed to ester formation at C-1. Whereas ester formation at C-1 should cause such a shift, hemi-ortho esters should shift in the opposite direction, *e.g.*, HCO_2CH_3, δ CH_3 = 3.79; $HOCH_3$, δ CH_3 = 3.47; and $HC(OCH_3)_3$, δ CH_3 = 3.33 (8). None of the NMR data reported previously (2) supports arguments against Structure I as the alternate form of III. In addition, the stability of a hemi-ortho ester (9)

Table I—Proton Chemical Shift Values (Parts per Million) for the Methyl Esters of I and III

Proton	Methyl Ester of I		Methyl Ester of III	
	Chloroform	Dimethyl Sulfoxide-d_6	Chloroform	Dimethyl Sulfoxide-d_6
Phenyl C-3 and C-5 2H	8.18–8.30 m	8.175, 8.27	8.18–8.30 m	8.155–8.24
Phenyl C-2 and C-6 2H	7.54–7.60 m	7.565, 7.65	7.54–7.66 m	7.61–7.695
NH	7.00–7.20 m	8.62 d (J = 9 Hz)	(7.0–7.2)	8.55 d
C-1 H	6.29 d (J = 6.5 Hz)	6.02 d (J = 5 Hz)	5.14 br s	5.05 br s
$CHCl_2$	5.95 s	6.47 s	5.81 s	6.64 s
C-2 H	4.20–4.60 m[a]	4.04–4.42 m	4.2–4.6 m[a]	4.04–4.42 m[a]
OCH_3	3.70 s	3.585	3.72 s	3.61 s
C-3 H	3.50–3.85 m	3.20–3.60	4.2–4.6 m[a]	4.04–4.42 m[a]
C-3 OH	4.2–4.6 m[a]	5.10 t (J = 5 Hz)	—	—
C-1 OH	—	—	?	6.24 d (J = 4.5 Hz)
CH_2CH_2	2.72 s	2.67 m	2.69 s	2.57 s

[a] Two unresolved protons were detected within this shift range.

Table II—^{13}C-NMR Spectra of Chloramphenicol, III, and I [a]

Carbon	Chemical Shift		
	Chloramphenicol	III	I[a,b]
C-1 (CHO)	69.02 (d)	69.51 (d)	72.75 (d)
C-2 (CHN)	56.79 (d)	53.52 (d)	54.94 (d)
C-3 (CH_2O)	60.27 (t)	63.13 (t)	59.67 (t)
Phenyl C-1 (C)	151.18 (s)	150.14 (s)	145.52* (s)
Phenyl C-2 and C-6 (CH)	127.29 (d)	127.42 (d)	127.64 (d)
Phenyl C-3 and 3-5 (CH)	122.84 (d)	122.85 (d)	123.22 (d)
Phenyl C-4 (CNO_2)	146.44 (s)	146.62 (s)	146.98* (s)
Amide C-2 (C=O)	163.38 (s)	163.64 (s)	163.51 (s)
Amide-2 ($CHCl_2$)	66.43 (d)	66.24 (d)	66.37 (d)
Succinyl C-1 (CO_2R)		171.84 (s)	171.18 (s)
Succinyl C-2 (CH_2)		28.60 (t)	28.80+ (t)
Succinyl C-3 (CH_2)		28.60 (t)	28.53+ (t)
Succinyl C-4 (CO_2H)		173.29 (s)	173.21 (s)

[a] Obtained in dimethyl sulfoxide-d_6; values are in parts per million from tetramethylsilane; letters in parentheses refer to peak character in off-resonance decoupled spectrum (s, singlet; d, doublet; and t, triplet). [b] The *, + indicate that these pairs of assignments may be reversed.

under the conditions used to isolate II by Sandmann *et al.* is questionable.

Finally, ^{13}C-NMR (a technique unavailable to Sandmann *et al.* at the time of their investigation) was used to verify the structure of I. The carbon chemical shifts of chloramphenicol and its two succinate derivatives are shown in Table II. The peaks near 163.5 and 66.3 ppm are consistent through the three spectra and are assigned as the carbonyl and methine carbons of the dichloroacetamide moiety, respectively. These shifts are quite consistent with shifts reported by Stothers (10) of 169.7 and 63.8 ppm for dichloroacetic acid and 165.0 and 69.7 ppm for dichloroacetyl chloride.

The assignment of the phenyl carbon shifts for the compounds of this study is based on the shifts of the equivalent carbons of *p*-nitrostyrene (C-1 = 143.6, C-2 and C-6 = 126.0, C-3 and C-5 = 123.1, and C-4 = 146.5 ppm) reported by Dhami and Stothers (11). The upfield shift of C-1 of the phenyl group of I relative to the other two compounds is due to the greater substitution at C-1 of the side chain in I. The assignments of C-1, C-2, and C-3 of the side chain are based on chemical shift and off-resonance decoupling results. The 2-carbon is at the highest field and shows a slight upfield shift on acylation (γ-substitution) at either C-1 or C-3. The 3-carbon is a triplet under off-resonance decoupling conditions. The 1- and 3-carbons show the expected downfield shifts on acylation. The succinyl moiety is assigned on the basis of the succinic acid shifts (CH_2 = 30.0 and CO_2H = 176.4 ppm) reported by Stothers (10). The carboxyl carbons are assigned at a lower field than the ester carbons, as is normally the case.

Most pertinent to the central argument of this paper are the shifts of the succinyl ester carbons at 171.84 and 171.18 ppm. The great similarity of these shifts argues that I is a normal ester, as is III. Furthermore, although reports of chemical shifts for hemi-ortho esters such as II are very rare due to their chemical instability, two such compounds are reported (12) as having shifts of a hemi-orthoacetate carbon at 77.1 ppm. This field shift is surprisingly high and may not be typical, but the ortho ester carbon of ethyl orthoformate absorbs at 112 ppm and those of two orthoacetates absorb at 118.1 and 119.5 ppm (12). It is unreasonable to suppose that the hemi-ortho ester carbon of II absorbs at 171 ppm, which is typical of a normal ester carbonyl carbon.

EXPERIMENTAL

Materials—The equilibrium mixture of III and its alternate form was obtained by dissolving 600 mg of chloramphenicol-3-monosuccinate[2] in 3 ml of water, adjusting the pH to 7.5 with 0.1 *N* NaOH, and allowing the resulting solution to stand at ambient temperature overnight.

Equipment—HPLC was carried out using a reversed-phase column[3] coupled to a UV detector[4] set at 254 nm.

Both the double-focusing mass spectrometer[5] and the NMR spectrometer[6] were coupled to data systems.

Procedures—One hundred microliters of the equilibrium mixture was injected onto the preparative reversed-phase column[5]. The components of the mixture were separated by isocratic elution using 0.01 *M* NaH_2PO_4–40% acetonitrile (final pH adjusted to 2.5 with phosphoric acid) as the mobile phase. A flow rate of 1 ml/min was used for analytical runs, and a rate of 2 ml/min was used for preparative runs. The eluate for each component was extracted with ethyl acetate and dried with magnesium sulfate. The volume of ethyl acetate was reduced by rotary evaporation, and the products were stored at −10° under a nitrogen atmosphere until they were analyzed.

Methylations were carried out by reaction with excess diazomethane[7] in ether. Trimethylsilylation was accomplished by adding 200 μl of *N,O*-bis(trimethylsilyl)acetamide in dimethylformamide[8] (2.5 mg/ml) to a solution of the target compound in acetonitrile.

REFERENCES

(1) F. H. Schmidt and W. Vömel, *Klin. Wochenschr.*, **43**, 535 (1965).

(2) B. Sandmann, D. Szulczewski, J. Windheuser, and T. Higuchi, *J. Pharm. Sci.*, **59**, 427 (1970).

(3) J. T. Burke, W. A. Wargin, and M. R. Blum, *ibid.*, **69**, 909 (1980).

(4) M. Margosis, *ibid.*, **59**, 501 (1970).

(5) G. Janssen and H. Vanderhaeghe, *J. Chromatogr.*, **82**, 297 (1973).

(6) T. Nakagawa, M. Mesada, and T. Uno, *ibid.*, **111**, 355 (1975).

(7) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden Day, San Francisco, Calif., 1962, pp. 43–45.

(8) C. J. Pouchert and J. R. Campbell, Aldrich Library of NMR Spectra, Aldrich Chemical Co., Milwaukee, Wis., 1974.

(9) M. Ahmad, R. G. Bergstrom, M. J. Cashen, Y. Chiang, A. J. Kresge, R. A. McClelland, and M. F. Powell, *J. Am. Chem. Soc.*, **101**, 2669 (1979).

(10) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic, New York, N.Y., 1972.

(11) K. S. Dhami and J. B. Stothers, *Can. J. Chem.*, **43**, 510 (1965).

(12) D. A. H. Taylor, *J. Chem. Soc., Perkin Trans. I*, **1974**, 437.

ACKNOWLEDGMENTS

The authors thank Ms. J. Sabatka for assistance with the HPLC.

[2] Obtained from Parke-Davis Co. as chloramphenicol sodium succinate (lot 391769A).

[3] A Bio Rad Bio Sil ODS 10, 25-cm × 4-mm column was used for analytical qualitative analysis. A Waters Associates μBondapak C_{18}, 30-cm × 7.8-mm column was used for preparative work.

[4] Waters Associates model 450 variable-wavelength detector.

[5] Varian MAT 731.

[6] Varian XL-100.

[7] Made by the reaction *p*-tolylsulfonylmethylnitrosamide (Diazaid, Aldrich Chemical Co.) with base.

[8] TRI-SIL/BSA formula D, Pierce Chemical Co.