The structure of these complexes is under investigation. Tin–metal bonding has recently been proposed by Gorsich[10] for the related compounds $Mo(CO)_5SnCl_3$ and π-$C_5H_5Fe(CO)_2SnCl_3$. The platinum–tin complexes may contain $(SnCl_3)^-$ ligands attached through platinum–tin bonds.

Acknowledgments.—R. V. L. wishes to acknowledge many helpful discussions with Professors Dietmar Seyferth and J. W. Irvine, Jr., during that part of this investigation conducted at the Massachusetts Institute of Technology.

(10) R. D. Gorsich, *J. Am. Chem. Soc.*, **84**, 2486 (1962).

CONTRIBUTION NO. 845 R. D. CRAMER
CENTRAL RESEARCH DEPARTMENT E. L. JENNER
EXPERIMENTAL STATION R. V. LINDSEY, JR.
E. I. DU PONT DE NEMOURS AND CO. U. G. STOLBERG
WILMINGTON, DELAWARE
DEPARTMENT OF CHEMISTRY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE, MASSACHUSETTS

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TIN(II) CHLORIDE COMPLEXES OF PLATINUM METALS

Sir:

It has been known for decades[1] that certain platinum metal halides or halide complex ions in aqueous solution or in solvents such as ethanol or ethyl acetate give colored species when treated with tin(II) chloride. The platinum chloride–tin(II) chloride system is also known to be a carbonylation and hydrogenation catalyst.[2]

Although Ayres[3] considered the platinum species to be cationic, *viz.*, $[PtSn_4Cl_4]^{4+}$, Shukla[1] showed by electrophoresis measurements that the rhodium and platinum species are anionic in hydrochloric acid solution, an observation we have confirmed by ion exchange study. Recently[4] a ruthenium complex (Ru:Sn ratio 1:4–5) was shown to be anionic and to be precipitated by large cations.

Our studies on the rhodium(III) chloride–tin(II) chloride system provided the first evidence for what we now consider to be the true nature of these tin-containing platinum metal complexes. Thus in ethanolic or in 3 *M* hydrochloric acid solution (main species $SnCl_3^-$) the Job method using the visible absorption spectrum showed that in both media the ratio Rh:Sn required for the formation of the red complex was 1:3. Addition of tetramethylammonium chloride to the solutions gave orange-yellow precipitates. The analysis corresponds to $[(CH_3)_4N]_4[Rh_2Sn_4Cl_{14}]$ [*Anal.* Calcd. C, 13.0; H, 3.2; Cl, 33.7; N, 3.8; Sn, 32.2. Found: C, 13.1; H, 2.9; Cl, 34.0; N, 3.7; Sn, 32.0] so that the interaction in 3 *M* acid may be written

$$2RhCl_3 + 6SnCl_3^- = [Rh_2Sn_4Cl_{14}]^{4-} + 2SnCl_5^-$$

We have prepared similar diamagnetic tetraalkylammonium salts (shown to be 2:1 electrolytes in dimethylformamide) of the ions $[Ru(SnCl_3)_2Cl_2]^{2-}$, $[Ir(SnCl_3)_2Cl_3]^{2-}$ and $[Pt(SnCl_3)_2Cl_2]^{2-}$. While the latter ion can be obtained from $PtCl_4^{2-}$ by chloride ion displacement, it is also formed by direct replacement by tin(II) chloride of the ligands in complexes such as $[C_2H_4PtCl_3]^-$ and mesityloxideplatinum(II) chloride.[5]

(1) For references see S. K. Shukla, *Ann. Chim.* (Paris), [13] **6**, 1383 (1961).

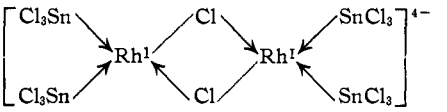
(2) E. L. Jenner and R. V. Lindsey, Jr., U.S. Pat. 2,876,254, March 3, 1959; R. D. Cramer, E. L. Jenner, R. V. Lindsey, Jr., and U. G. Stolberg, *J. Am. Chem. Soc.*, **85**, 1691 (1963).

(3) G. H. Ayres, B. L. Tuffy and J. S. Forester, *Anal. Chem.*, **27**, 1742 (1955); A. S. Meyer and G. H. Ayres, *J. Am. Chem. Soc.*, **77**, 2671 (1955).

(4) H. Okuno, H. Yamatera, T. Ishimori and K. Mizumachi, Abstract 4E3, 7th International Conference on Coördination Chemistry, 1962.

(5) G. W. Parshall and G. Wilkinson, *Inorg. Chem.*, **1**, 896 (1962).

Although numerous examples of transition metal to tin bonds are known from the work of Hieber and more recently Gorsich,[6] these are best regarded as derivatives of tin(IV). However, we believe that the present complexes are best formulated as complexes of tin(II). Thus, for example, the rhodium and platinum species can be considered as square planar complexes of Rh^I and Pt^{II}, respectively, in which either $SnCl_3^-$ (in the complex anions) or solvated tin(II) chloride (in neutral species) act as donor ligands using the lone-pairs known to be present.[7] The ion $[Rh_2Sn_4Cl_{14}]^{4-}$ thus can be regarded as



and the other species can be similarly formulated. Support for this view is provided by the direct replacement, from the Ru, Rh, Ir and Pt complexes, of tin(II) chloride—which can be characterized by standard tests—by ligands such as pyridine, triphenylphosphine and carbon monoxide, in the latter case the rhodium complex giving $[Rh(CO)_2Cl]_2$. In these studies we have also isolated an orange complex which can be formulated $[(C_6H_5)_3P]_2PtClSnCl_3$ and there appear to be similar complexes of rhodium, iridium and ruthenium. It may be noted that Dwyer and Nyholm[8] have reported compounds of rhodium containing methyl diphenylarsine and stannous chloride which they formulated as bridged species containing combinations of Sn–Cl–Rh, Sn–Cl–Sn and Rh–Cl–Rh bridges; we believe that Rh–Sn bonds probably are present here also.

(6) R. D. Gorsich, *J. Am. Chem. Soc.*, **84**, 2486 (1962), and references therein.

(7) D. Grdenić and B. Kamenar, *Proc. Chem. Soc.*, 304 (1961); *J. Inorg. Nucl. Chem.*, **24**, 1039 (1962).

(8) F. P. Dwyer and R. S. Nyholm, *J. Proc. Roy. Soc. N. S. Wales*, **76**, 129 (1942).

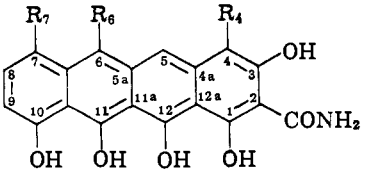
INORGANIC CHEMISTRY RESEARCH LABORATORIES A. G. DAVIES
IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY G. WILKINSON
LONDON, S. W. 7, ENGLAND J. F. YOUNG

RECEIVED MARCH 27, 1963

BIOSYNTHESIS OF THE TETRACYCLINES. V.[1] NAPHTHACENIC PRECURSORS

Sir:

We wish to report the biological conversion of 1,3,10,11,12-pentahydroxynaphthacene-2-carboxamide[2] (**2**) and some related compounds to tetracycline antibiotics. These naphthacenic precursors, which can be represented by the general formula **1**, were prepared by degradation of tetracycline antibiotics.[3]



1

2 R_4, R_6, R_7 = H
3 R_4, R_7, = H; R_6 = CH_3
4 R_4 = $N(CH_3)_2$; R_6 = CH_3; R_7 = H
5 R_4 = $N(CH_3)_2$; R_6 = H; R_7 = Cl

(1) Paper IV: J. R. D. McCormick, P. A. Miller, S. Johnson, N. Arnold and N. O. Sjolander, *J. Am. Chem. Soc.*, **84**, 3023 (1962).

(2) We propose the name "pretetramid" for this tetracyclic amide which is the parent of the family of precursors reported here.

(3) The preparation of compound **2** from 6-demethyltetracycline is described in the accompanying communication [J. R. D. McCormick, J. Reichenthal, S. Johnson and N. O. Sjolander, *J. Am. Chem. Soc.*, **85**, 1694 (1963)]. Compound **3** was prepared from tetracycline by the method of A.

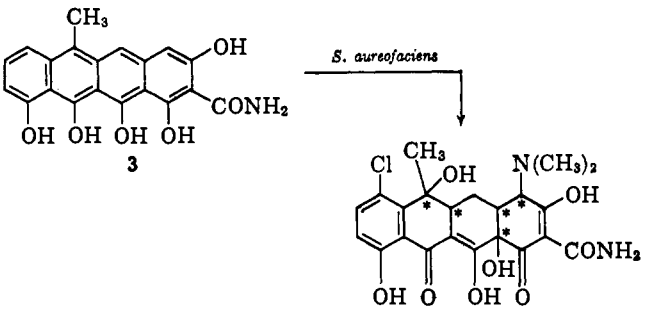
TABLE I
CHARACTERISTICS OF *S. aureofaciens* STRAINS USED

Strain	Descended from	Main biosynthetic pathway block	C-6 methylation block	Chlorination block
V828	7-Cl-TC[a] producer	Yes	No	No
ED1369	7-Cl-6-demethyl-TC producer	Yes	Yes	No
F198-1	6-Demethyl-TC producer	Yes	Yes	Yes

[a] TC = tetracycline.

The conversions were first demonstrated by the use of certain non-tetracycline-producing mutants of *Streptomyces aureofaciens*.[4] The naphthacenic precursors were dissolved in dimethyl sulfoxide containing 1% magnesium acetate, the solutions were added to shaker flask fermentations 24 hr. after inoculation, and the incubation was continued an additional 96 hr. The fermented mashes were assayed for antibiotic content by the agar diffusion method, and the antibiotic products were identified by paper chromatography.

atom of chlorine for hydrogen at C-7 and of the elements of a dimethylamino group for an atom of hydrogn at C-4. *It should also be noted that all five of the asymmetric centers of the tetracycline molecule are generated in the course of these transformations.*



3

7-Chlorotetracycline

The sequence and detailed mechanisms by which these transformations occur are still not known, except that the 5a,6-hydration[1] can occur as the net result of a

TABLE II
TETRACYCLINE ANTIBIOTICS PRODUCED FROM NAPHTHACENIC PRECURSORS

S. aureofaciens strain: Precursor added[a]	V828	ED1369	F198-1
	Product[b] observed (μg./ml.[c])		
None	None (<1)	None (<1)	None (<1)
2	7-Cl-6-demethyl-TC[d] (80)	7-Cl-6-demethyl-TC (85)	6-Demethyl-TC (15)
3	7-Cl-TC (420)	7-Cl-TC (190)	TC (110)
4	7-Cl-TC (60)	7-Cl-TC (45)	TC (25)
5	7-Cl-6-demethyl-TC (30)	7-Cl-6-demethyl-TC (40)	7-Cl-6-demethyl-TC (30)

[a] Ten milligrams of compound was dissolved under nitrogen in 1 ml. of dimethyl sulfoxide containing 1% magnesium acetate and added to 25 ml. of the 24-hr.-old fermented mash to give a precursor concentration of 400 μg./ml. [b] The identities of the products were established by paper chromatography. [c] Microbiological assay at end of fermentation. Addition of precursors at the end of the fermentation resulted in no antibacterial activity. [d] TC = tetracycline.

The mutants used were selected for the characteristic metabolic blocks shown in Table I. The data in Table II show that chlorinated tetracyclines result from the use of either a chlorinated precursor (**5**) or a chlorinating mutant (V828 or ED1369), while non-chlorinated tetracyclines arise only from the use of non-chlorinated precursors (**2**, **3**, or **4**) *and* a non-chlorinating mutant (F198-1). Conversely, 6-methylated tetracyclines arise only from 6-methylated precursors (**3** or **4**), whether methylating (V828) or non-methylating mutants (ED1369 or F198-1) are used for the conversion. These observations indicate that, in the normal biosynthesis of the tetracyclines, the 6-methyl group is introduced before the tetracyclic amide is elaborated and show that the 7-chloro substituent is introduced after this point.

Conversion yields ranged from 3 to 75% of theory, depending on the strain and precursor combination used. The highest conversion was achieved by the addition of **3** to strain V828; the identity of the product was confirmed, in this instance, by isolation of crystalline 7-chlorotetracycline in 7.5% over-all yield.

The conversion of **3** to 7-chlorotetracycline involves the net addition of the elements of two moles of water (at 4a, 12a and at 5a, 6), and the substitution of an penultimate 6-hydroxylation[1] and a final 5a,11a-reduction.[5] It is significant, however, that all of these biosynthetic steps are now shown to be accomplished *after* the generation of the naphthacene moiety, and the usefulness of this conversion system in the further detailed study of these steps is clearly indicated.

The participation of **2** as in intermediate in the biosynthesis of 6-demethyltetracycline is additional confirmation of the probable polyacetate[6] origin of the tetracyclines as originally suggested by Robinson.[7]

Although terrarubein (**4**) would appear to be a reasonable intermediate between **3** and the tetracyclines, our experiments suggest that this is not the case. Specifically, the conversion yield from terrarubein to tetracycline or to 7-chlorotetracycline consistently has been much less than the yield from **3**; and experiments[8] involving the addition of C^{14}-methyl-labeled methionine with **4** or **5** to these *S. aureofaciens* fermentations have shown extensive incorporation of C^{14}-methyl groups into the dimethylamino group of the tetracy-

Green, R. G. Wilkinson and J. H. Boothe, *J. Am. Chem. Soc.*, **82**, 3946 (1960). Terrarubein (**4**) was prepared from tetracycline by the method of R. K. Blackwood, H. H. Rennhard and C. R. Stephens, *ibid.*, **82**, 745 (1960), and **5** was similarly prepared from 7-chloro-6-demethyltetracycline by way of the previously undescribed 12a-formate ester (not isolated) and 4a,12a-anhydro-7-chloro-6-demethyltetracycline hydrochloride (*Anal.* Found for $C_{21}H_{19}N_2O_7Cl \cdot HCl \cdot H_2O$: C, 50.02; H, 4.31; N, 5.62). The dehydration of this substance to **5** was carried out in *p*-chlorophenol solution by the use of anhydrous hydrogen bromide in acetic acid. The product, 7-chloro-4-dimethylamino-1,3,10,11,12-pentahydroxynaphthacene-2-carboxamide (**5**), crystallized as the hydrobromide with a molecule of *p*-chlorophenol of solvation. *Anal.* Found for $C_{21}H_{17}N_2O_8Cl \cdot C_6H_5OCl \cdot HBr$: C, 51.4; H, 3.61; N, 4.39; HBr, 10.8.

(4) The *S. aureofaciens* mutants were originally selected by Dr. R. Weindling, Dr. J. A. Growich and Miss Ursula Hirsch of these Laboratories.

(5) J. R. D. McCormick, N. O. Sjolander, P. A. Miller, U. Hirsch, N. Arnold and A. P. Doerschuk, *J. Am. Chem. Soc.*, **80**, 6460 (1958).

(6) In recent years the original polyacetate hypothesis advanced by J. N. Collie [*J. Chem. Soc.*, **91**, 1806 (1907)], to account for the biosynthesis of certain natural products has been refined and modified to the current polymalonate hypothesis. Specifically, F. Lynen [*J. Cellular Comp. Physiol.*, Suppl. 1, **54**, 33 (1959)] has now suggested that malonyl coenzyme A is the actual condensing unit in the polyacetate series of natural products; and in recent papers, S. J. Wakil [*J. Am. Chem. Soc.*, **80**, 6465 (1958)], K. Mosbach [*Naturwiss.*, **48**, 525 (1961)] and S. Gatenbeck [*Biochem. Biophys. Res. Commun.*, **6**, 422 (1961)] have presented experimental evidence for the role of malonyl coenzyme A in the biosynthesis of fatty acids, orsellinic acid, and 5-hydroxytetracycline, respectively.

(7) R. Robinson, "Structural Relations of Natural Products," Oxford Press, 1955, p. 58. The nonaacetyl derivative, 1,3,10,11,12-pentahydroxynaphthacene, was suggested by Robinson as an intermediate in the biosynthesis of the tetracyclines before any experimental information on this subject was available.

(8) Unpublished work carried out with the coöperation of Mr. E. R. Jensen.

clines produced. We therefore feel that the dimethylaminonaphthacenes, **4** and **5**, are not true intermediates in the biosynthesis of the tetracyclines but may be accessible to the biosynthetic pathway by loss of the dimethylamino group.[9]

It would appear from the high yield achieved in the conversion of **3** to 7-chlorotetracycline, in spite of the number and complexity of the steps involved, that **3** is a normal intermediate[10] in the biosynthesis of the 6-methylated tetracyclines. By analogy we assume that **2** plays a similar role in the biosynthesis of the 6-nonmethylated tetracyclines.

(9) While incorporation of C^{14} methyl groups into the tetracyclines could be accounted for by exchange of methyls with methionine, we have not observed such exchange in a closely related system; namely, in the biological rehydration of 5a,6-anhydro-7-chlorotetracycline by *S. aureofaciens* V828 in the presence of C^{14}-methyl-labeled methionine we obtained 7-chlorotetracycline containing no C^{14}.

(10) As the usual simplification, we consider the isolated compounds to be the equivalent of their conjugated or enzyme-complexed forms which would presumably be involved in the biosynthetic processes as true intermediates.

LEDERLE LABORATORIES
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK
J. R. D. MCCORMICK
SYLVIA JOHNSON
NEWELL O. SJOLANDER

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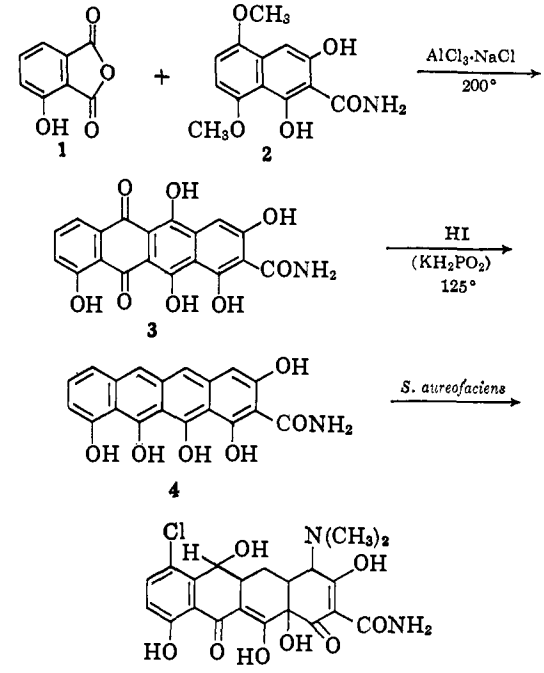
BIOSYNTHESIS OF THE TETRACYCLINES. VI.[1] TOTAL SYNTHESIS OF A NAPHTHACENIC PRECURSOR: 1,3,10,11,12-PENTAHYDROXYNAPHTHACENE-2-CARBOXAMIDE

Sir:

We have reported previously[1] the biological conversion by *Streptomyces aureofaciens* of 1,3,10,11,12-pentahydroxynaphthacene-2-carboxamide (**4**) and several related compounds to tetracycline antibiotics. In that work the naphthacene derivatives were prepared from tetracycline antibiotics by known degradation methods. An attempted synthesis of one of these compounds, 6-methyl-1,3,10,11,12-pentahydroxynaphthacene-2-carboxamide, has been reported,[2a,b] but to date no total synthesis of an intermediate biologically convertible to a tetracycline has been reported.[3] We now wish to report the total synthesis of **4** and the biological conversion of the synthetic substance to 7-chloro-6-demethyltetracycline.

The synthesis of **4** was accomplished by condensing 3-hydroxyphthalic anhydride[4] (**1**) with 1,3-dihydroxy-5,8-dimethoxynaphthalene-2-carboxamide[5] (**2**) and reducing the resulting naphthacenequinone (**3**).

The condensation of **1** with **2** was accomplished by fusion of an intimate mixture of 2 mmoles of each with 10 g. of anhydrous aluminum chloride and 2 g. of sodium chloride at 200° for 2 hr. The melt was cooled, decomposed with 3 *N* hydrochloric acid and the resulting black crude solid was washed thoroughly with water. The desired product, 1,3,5,10,12-pentahydroxynaphthacene-6,11-quinone-2-carboxamide (**3**) was separated by dissolving the crude solid in 1:1 dimethylformamide–water and extracting into 1:10 triethylamine–chloroform. The quinone (146 mg., 19%) was isolated by evaporating the deep blue extract. It was recrystallized from dimethylformamide–triethylamine. *Anal.* Found for $C_{19}H_{11}NO_8$: C, 59.4; H, 3.06; N, 3.54; m.p. 355–360° dec.; λ_{max} in mμ (ϵ): 303 (23,400), 384 (4,220), 404 (5,070), 578 (13,900), 627 (25,900).[6]



7-Chloro-6-demethyltetracycline

The quinone was reduced by dissolving the total product from the preceding step in 5 ml. of phenol, adding 2 ml. of 58% hydriodic acid and 200 mg. of potassium hypophosphite, and refluxing for 3 hr. The product crystallized on cooling to form 1,3,10,11,12-pentahydroxynaphthacene-2-carboxamide.[7] *Anal.* Found for $C_{19}H_{13}NO_8$: C, 65.2; H, 3.92; N, 3.92; m.p. 290–320° dec., λ_{max} in mμ (ϵ): 231 (23,200), 264 (28,900), 282 (31,100), 337 (15,800), 394 (16,100), 490 (16,400).[6] The infrared spectrum (KBr disk) was identical with that of authentic **4**.

Authentic **4** was prepared from 6-demethyltetracycline following the procedure used by Green, Wilkinson and Boothe[8] on tetracycline. 6-Demethyltetracycline was reduced to 4-dedimethylamino-6-demethyl-12a-deoxytetracycline with zinc and acetic acid. The isolated crude product [*Anal.* Found for $C_{19}H_{17}NO_7$: C, 61.1; H, 5.00; N, 3.30; λ_{max} (0.1 *N* hydrochloric acid in methanol) in mμ (ϵ): 261 (18,600), 353 (12,000), 431 (3,030), 452 (shoulder) (2,350)] was brominated in tetrahydrofuran with N-bromosuccinimide and the resulting crude 12a-bromo derivative was dehydrobrominated in pyridine at 100° to yield 4a,12a-anhydro-4-dedimethylamino-6-demethyltetracycline. *Anal.* Found for $C_{19}H_{15}NO_7$: C, 61.2; H, 4.26; N, 3.65; λ_{max} (0.1 *N* hydrochloric acid in methanol) in mμ (ϵ): 273 (10,300), 402 (34,900), 420 (32,900). This product was dissolved in phenol and dehydrated with hydrochloric acid in acetic acid to yield crystalline **4**. *Anal.* Found for $C_{19}H_{13}NO_6$: C, 64.6; H, 3.91; N, 3.89; λ_{max} in mμ (ϵ): 232 (22,800), 264 (28,600), 281 (31,000), 333 (14,000), 394 (15,300), 490 (16,700).[6]

(1) Paper V: J. R. D. McCormick, S. Johnson and N. O. Sjolander, *J. Am. Chem. Soc.*, **85**, 1692 (1963).

(2) (a) Y. T. Huang, H. C. Tsung, L. H. Tai, H. Y. Sheng and T. Y. Tu, *Hua Hsüeh Hsüeh Pao*, **24**, 311 (1958); (b) Y. T. Huang, *Tetrahedron*, **11**, 52 (1960).

(3) J. H. Boothe and co-workers [*J. Am. Chem. Soc.*, **81**, 1006 (1959)] and H. Muxfeldt [*Chem. Ber.*, **92**, 3122 (1959)] have reported the total syntheses of 5a,6-anhydro-7-chloro-4-dedimethylamino-6-demethyl-12a-deoxytetracycline and 5a,6-anhydro-4-dedimethylamino-12a-deoxytetracycline, respectively. We have found that these compounds are not biologically converted to tetracycline antibiotics in our system (unpublished work).

(4) E. L. Eliel, A. W. Burgstahler, D. E. Rivard and L. Haefele, *J. Am. Chem. Soc.*, **77**, 5092 (1955).

(5) Z. Budesinsky and A. Svab, *Chem. Listy*, **51**, 1333 (1957).

(6) Absorption spectra were determined in 98% sulfuric acid containing 0.1% of boric anhydride. Solutions were allowed to stand 30 min. before spectra were determined.

(7) The condensation of 3-hydroxyphthalic anhydride with the naphthaleneamide would be expected to generate two isomeric products, a 7-hydroxynaphthacene and a 10-hydroxynaphthacene. Separation of these two isomers presumably occurred either in the triethylamine–chloroform extraction or in the crystallization to the final product.

(8) A. Green, R. G. Wilkinson and J. H. Boothe, *J. Am. Chem. Soc.*, **82**, 3946 (1960).