Although the hydrocellulose–cellulose column had previously been used successfully in our laboratory in the separation of a similar mixture, the results shown in Table III indicate that in this case it failed to resolve the various components. Probably, it was due to the deterioration of the column.

Analyses of Fraction F_1–F_5.—Fraction F_1 was hydrolyzed by refluxing with 1 *N* sulfuric acid (25 ml) for 5 hr. A major portion of the fraction (about 320 mg) which did not go in solution during hydrolysis was removed, methanolyzed with methanolic hydrogen chloride, and finally was subjected to acid hydrolysis; despite this treatment, the yellow oily product remained insoluble. Examination of this insoluble oily product by paper chromatography in solvent C showed that it moved with the solvent front as a yellow spot and did not give a color with either spray reagent E or F. It was also found to be optically inactive and did not reduce Fehling's solution. These facts point to the noncarbohydrate nature of the material and so it was discarded.

The hydrolysate of the soluble portion (190.1 mg), by paper chromatography using solvent C and spray reagent E and F, showed the presence of 1-*O*-methyl-D-erythritol and traces of 1,4-di-*O*-methylerythritol, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose. The mixture was separated on paper into three fractions and the amount of each fraction was determined either by the phenol–sulfuric acid method or gravimetrically. The results are recorded in Table IV.

TABLE IV

COMPONENTS IN FRACTION F_1

Component	Wt,[a] mg
Crystalline 2,3,4,6-tetra-*O*-methyl-D-glucose	3.3 (p)
Crystalline 2,3,6-tri-*O*-methyl-D-glucose	94.7 (g)
1-*O*-methyl-D-erythritol	76.8 (g)

[a] g, gravimetrically; p, phenol–sulfuric acid method.

Fraction F_2 (254.2 mg) was hydrolyzed for 5–6 hr with 1 *N* sulfuric acid (10 ml). The hydrolysate afforded a syrupy residue (231 mg) which consisted of 2,3,6-tri-*O*-methyl-D-glucose and 1-*O*-methyl-D-erythritol as shown by paper chromatography using solvent C and spray reagents E and F. The amounts of 2,3,6 tri-*O*-methyl-D-glucose and 1-*O*-methyl-D-erythritol obtained by preparative paper chromatography were 26 mg and 195 mg, respectively.

Fraction F_3 (77.5 mg) was hydrolyzed by refluxing with 1 *N* hydrochloric acid (5 ml) for 5–6 hr. The syrupy product (68 mg) was shown by paper chromatography in solvent C using spray reagents E and F to contain 2,3,6-tri-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, 2,6-di-*O*-methyl-D-glucose, and 1-*O*-methyl-D-erythritol. The mixture was separated on paper into three fractions: (a) 2,3,6-tri-*O*-methyl-D-glucose (14.7 mg), (b) a mixture of 2,3-di-*O*-methyl-D-glucose and 1-*O*-methyl-D-erythritol (44 mg), and (c) 2,6-di-*O*-methyl-D-glucose (1.8 mg). The amount of 2,3-di-*O*-methyl-D-glucose in b, determined by the phenol–sulfuric acid method, was 1.4 mg.

After hydrolysis, fraction F_4 (142.2 mg) was identified as 1-*O*-methyl-D-erythritol by paper chromatography using solvent C and spray reagents E and F.

Fraction F_5 (11.1 mg) was hydrolyzed with 1 *N* sulfuric acid (5 ml) by heating in a boiling-water bath for 5–6 hr. Examination of the resulting syrupy residue by paper chromatography in solvent C using spray reagents E and F revealed the presence of mono-*O*-methyl-D-glucose (R_f 0.05). The residue was dissolved in water (50 ml) and the amount of mono-*O*-methyl-D-glucose determined as glucose by the phenol–sulfuric acid method was 1.2 mg.

Identification of Methylated Glucose Derivatives.—The various methylated glucose derivatives including 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3-di-*O*-methyl-D-glucose were characterized as reported earlier.[29] Component 2,6-di-*O*-methyl-D-glucose was found to be chromatographically identical, in two solvent systems C and D, with an authentic specimen. It gave 2,6-di-*O*-methyl-D-glucose 1,3,4-tris(*p*-azobenzoate) with *p*-phenylazobenzoyl chloride in pyridine, mp 203–205°, lit.[35] mp 205–207°.

Acknowledgment.—This work was supported in part by the National Science Foundation, the National Institutes of Health, the Northern Utilization Research and Developmnt Division (U. S. Department of Agriculture), and the Corn Industries Research Foundation.

(35) K. Freudenberg and G. Hill, *Ber.*, **74B**, 237 (1941).

The Structural Characterization of Tetrangomycin and Tetrangulol

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Two new benz[*a*]anthraquinone derivatives have been isolated from fermentations of *Streptomyces rimosus*. Experiments are reported which lead to the assignment of structure **1** to tetrangomycin and **3** to tetrangulol.

Despite the intensive examinations of microbial metabolites carried out in recent years and the remarkably diverse structural types already uncovered, new types are coming to light with increasing frequency.[1] We would like to describe two metabolites belonging to a new structural type which were discovered in the course of our antibiotic screening program. These compounds, tetrangomycin and tetrangulol, represent the first recorded isolation of benz[*a*]-anthraquinone derivatives from a living system. The finding is particularly intriguing in light of the well-known carcinogenic properties of hydrocarbons of this ring type.[2]

Tetrangomycin (**1**) was isolated from cultures of a variant strain of *Streptomyces rimosus* as an optically active, yellow quinone melting at 182–184° and having the molecular formula $C_{19}H_{14}O_5$ (M = 322 ± 0).[3,4] The ultraviolet and visible absorption spectra were similar to those reported for 1-hydroxyanthraquinone.[5] The infrared spectrum is consistent with an aromatic quinone in which one of the quinone carbonyls is chelated (1642 cm^{-1}) and the other is not (1678 cm^{-1}).[6] A third carbonyl band is present whose frequency (1705 cm^{-1}) is intermediate between that of an aromatic-conjugated ketone (1690 cm^{-1}) and a noncon-

(1) J. H. Richards and J. B. Hendrickson, "The Biosynthesis of Steroids, Terpenes and Acetogenins," W. A. Benjamin, Inc., New York, N. Y., 1964.

(2) B. Pullman, "The Modern Theory of Molecular Structure," Dover Publications, Inc., New York, N. Y., 1962, pp 69–71, and references therein.

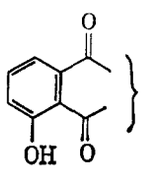
(3) The molecular weight was determined mass spectrometrically, using an Atlas CH4 instrument with a direct inlet system.

(4) A preliminary report emphasizing the biological properties of these metabolites was presented before the International Congress of Chemotherapy at Washington, D. C., Oct 21, 1965, and will appear in *Antimicrobial Agents Chemotherapy*–1965.

(5) T. Yoshimoto, *J. Chem. Soc. Japan*, **84**, 733 (1963).

(6) H. Bloom, L. H. Briggs, and B. Cleverley, *J. Chem. Soc.*, 178 (1959).

jugated ketone (1715 cm^{-1}).[7] The nmr spectrum (Figure 1) of tetrangomycin contained an unsplit signal at τ 8.53 attributable to a methyl group on a carbon bearing an electronegative substituent. Also found were two unsplit signals at τ 6.87 and 7.00, attributable to two aliphatic methylene groups which are insulated from each other and other hydrogens. The aromatic region contained signals for five hydrogens, two of which appear as an AB quartet (τ 2.52 and 1.87; J = 8 cps). The magnitude of the coupling constant indicates these two hydrogens to be situated *ortho* to one another, and the lack of additional coupling indicates them to be isolated from other hydrogens. The pattern for the remaining three aromatic hydrogens (τ 2.2–2.9) is very similar to the aromatic hydrogen pattern observed in ϵ-rhodomycinone,[8] which contains structural fragment **2**.



2

In addition to the above-mentioned signals, the nmr of tetrangomycin contained two deuterium exchangeable peaks at τ 5.75 and −2.08, which are due to a free and a hydrogen-bonded hydroxyl function, respectively. Additional evidence on this point was provided by acetylation of tetrangomycin, which gave, as the major product, an optically active monoacetate. The nmr spectrum of this product is similar to that of tetrangomycin except for the loss of the strongly hydrogen-bonded hydroxyl hydrogen adsorption (τ −2.08), the gain of an acetate methyl peak (τ 7.60), and slight alterations in the portion of the aromatic pattern attributed to structural increment **2**. The infrared spectrum of tetrangomycin acetate showed the expected carbonyl absorption pattern, with bands at 1770 cm^{-1} (phenolic acetate), 1705 cm^{-1} (ketone), and 1685 cm^{-1} (*nonchelated* quinone carbonyl). A small amount of an *optically inactive* diacetate was also isolated. The significance of this substance will be discussed below.

It was anticipated that the visible absorption spectrum of tetrangomycin would undergo a bathochromic shift in the presence of alkali, because of its phenolic properties, and, in fact, a shift from 400 to 477 mμ was observed. However, reacidification did not reconvert the spectrum to its original shape, and a new maximum was observed at 425 mμ in acidic or neutral solutions. This strongly suggested facile transformation of tetrangomycin to a new substance under mildly alkaline conditions. When tetrangomycin was treated with dilute alkali for a short time on a preparative scale, a new, optically inactive quinone was isolated. This new substance, designated tetrangulol, is coproduced along with tetrangomycin by the organism, and had been previously isolated from culture filtrates of the same fermentation. From the facile alkaline conversion of tetrangomycin to tetrangulol, it was readily apparent that they were closely related structurally, and the

(7) K. Nakanishi, "Infrared Absorption Spectroscopy, Practical," Holden-Day, Inc., San Francisco, Calif., 1962, p 42.

(8) H. Brockmann, *Fortsch. Chem. Org. Naturstoffe*, **21**, 122 (1963).

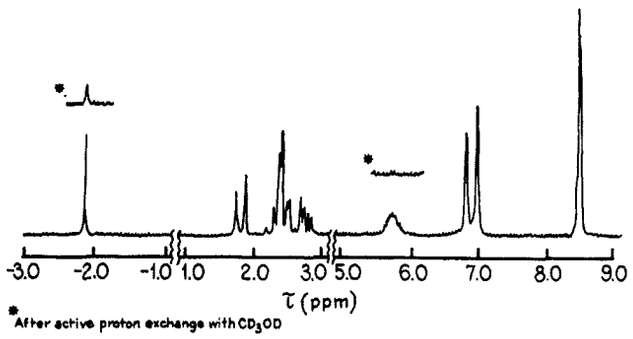


Figure 1.

physicochemical properties which we had obtained for tetrangulol were very pertinent to the structure characterization of both microbial metabolites.

Tetrangulol (**3**), $C_{19}H_{12}O_4$, shows the well-known oxidation–reduction behavior of quinones with sodium hydrosulfite.

Its molecular formula differs from that of tetrangomycin by lacking the elements of water. The infrared spectrum of tetrangulol indicates, in contrast to tetrangomycin, that *both* quinone carbonyls are chelated (1640 and 1625 cm^{-1}). In addition, the carbonyl band at 1705 cm^{-1} of tetrangomycin is no longer present.

That the two nonquinone carbonyl oxygens are phenolic hydroxyl groups, and are responsible for the observed chelation, was shown by conversion of tetrangulol to a mono- and a di-O-methyl ether, and a diacetate. The pertinent infrared carbonyl frequencies of these derivatives are collected in Table I. These

TABLE I

INFRARED BANDS OF VARIOUS DERIVATIVES (KBR DISK)

Substance	Band, cm^{-1}			
Tetrangulol		1640, 1625	1585	1550
Diacetyltetrangulol	1670	1630 (m)	1595	
O-Methyltetrangulol	1650	1625	1585	1550
Di-O-methyltetrangulol	1660	1620 (m)	1590	1560
5,11-Dimethoxybenz[*a*]anthraquinone	1660	1615 (m)	1585	1560
Anthraquinone	1665			
Tetraacetyltetrangulol		1620 (w)	1580	1570
1-Hydroxyanthraquinone	1667	1634		
1,4- and 1,5-dihydroxyanthraquinone		1626		
1,8-Dihydroxyanthraquinone	1678	1617		

data show that the fully acetylated and alkylated derivatives of tetrangulol have quinone carbonyl bands at 1650 to 1670 cm^{-1}, which frequency range is characteristic of unchelated anthraquinone carbonyls.[6] There is a medium intensity aromatic band at about 1620 cm^{-1} in all the spectra in the tetrangulol series which complicates the picture somewhat, as it sometimes coincides with a chelated quinone band; but, as it is present even in the reductive acetylation product and in the synthetic model, it can be disregarded in this argument.

It is interesting to note that the minor product from the acetylation of tetrangomycin is identical with tetrangulol diacetate. Apparently, under the conditions of acetylation, a partial conversion of tetrangomycin to tetrangulol occurred due to the formal loss of the elements of water. This, then, was the second example of the facility of this transformation.

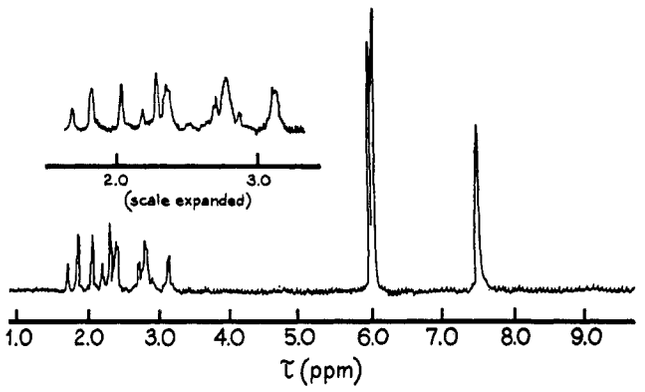


Figure 2.

The spectral evidence reported above defines the nature and something of the spatial interrelationships of the four oxygen functions in tetrangulol. Obviously the observed chelation requires that each hydroxyl be in the immediate vicinity of one of the quinone carbonyls in such a way as to hydrogen bond strongly with it. This will, as it will be seen, strictly limit the possible oxygenation sites in the two metabolites.

The nmr spectrum of di-O-methyltetrangulol (Figure 2) was very informative in defining the chemical nature of the transformation of tetrangomycin to tetrangulol. The absorption of the methyl group was shifted from τ 8.53 to 7.53 indicating that it was now on an aromatic ring. The methylene peaks had disappeared, being replaced by two new peaks attributed to two meta-situated aromatic protons (τ 2.85 and 3.18; $J \approx 2$ cps). The AB quartet was still present; however, one hydrogen of this absorption pattern was shifted from τ 2.52 to 2.20. These changes are rationalized best by loss of a molecule of water, and enolization of the nonquinone carbonyl of tetrangomycin resulting in the aromatization of the ring which previously contained the two insulated methylene groups and the aliphatic methyl group. The AB quartet is obviously close enough to the new aromatic ring to "feel" the newly generated ring current.

To complete the structural analysis it was now necessary to determine the nature of the eighteen carbons making up the carbon skeleton and place the functions thereon. The nature of the carbon skeleton was established on spectral and degradative grounds. In a substantial number of cases, Brockmann[9] has shown that polyacetoxyanthraquinones possess visible absorption spectra which are remarkably like that of anthraquinone itself. The main difference is a slight shift of the visible maxima to higher wavelength in the acetylated derivatives. Thus it was exciting to note that the spectrum of diacetoxytetrangulol was quite different from naphthacenequinone or anthraquinone but was very similar to that of benz[*a*]anthraquinone (Table II). Of particular significance is the very broad and detailed appearance of this region in the benz[*a*]-anthracene series when compared to the linear derivatives. These suggestive data were confirmed in detail upon zinc dust distillation of tetrangulol which produced a crystalline hydrocarbon whose ultraviolet absorption spectrum was clearly that of a benz[*a*]anthracene. Unfortunately the yield was so low that the product could not be recrystallized to constant melting point

(9) H. Brockmann and W. Müller, *Ber.*, **92**, 1164 (1959).

TABLE II

COMPARISON OF VISIBLE SPECTRA OF DIACETYLTETRANGULOL AND VARIOUS QUINONES

Substance	Visible maxima, mμ
Diacetyltetrangulol	400 sh, 360, 338 sh
Benz[*a*]anthraquinone	390 sh, 375 infl, 360, 333 sh
Anthraquinone	325
Naphthacenequinone	400, 321

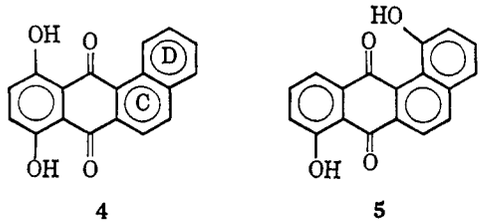
for definitive identification, but the data obtained were sufficient to exclude a number of the possibilities. Because of the interest in benz[*a*]anthracenes as chemical carcinogens, all of the possible monomethyl derivatives have been synthesized and their properties recorded in the literature.[10] The ultraviolet absorption spectra are very complex and those of many of the methyl benz[*a*]anthracenes differ from one another in small but definite ways. A comparison of those derivatives which are most like the tetrangulol product is given in Table III. The derivatives listed are those lacking additional peaks in the ultraviolet and which have melting points above 140 and below 170°. These hydrocarbons contain all the carbons of the metabolite, thus establishing its skeleton except for the final location of the methyl group.

TABLE III

COMPARISON OF TETRANGULOL HYDROCARBON AND SEVERAL BENZ[*a*]ANTHRACENES

Substance	Mp, °C	Ultraviolet spectrum, mμ
Tetrangulol hydrocarbon	146–148	384.5, sh 374, 358.5, 342, 328, 314, 300, 287, 277, 268, 256
3-Methyl-benz[*a*]-anthracene	160	385.5, 376, 360.5, 343.5, 329, 315, 301.5, 289, 278, 268.5, 258
9-Methyl-benz[*a*]-anthracene	151	385, sh 377, 360, 343.5, 330, 314.5, 301, 289, 279, 269.5, 257

Now that the carbon framework was known, except for the exact location of the methyl group, there remained primarily the task of locating the oxygen functions of tetrangulol and deciding which ring was aliphatic in tetrangomycin. The spectral evidence imposed some restrictions, and additional restrictions came from oxidative degradation studies. Treatment of tetrangulol with alkaline permanganate destroyed the oxygen-bearing rings and gave benzene-1,2,3,4-tetracarboxylic acid. This fragment apparently contains the two *ortho* hydrogens responsible for the observed AB quartet in the nmr spectrum. In a monomethylbenz[*a*]anthraquinone, regardless of the site of the methyl group, this fragment could only arise from ring C and furthermore eliminates the possibility of substitution in that ring. As ring D was destroyed, it is highly likely that it is oxygen bearing. In fact, the accumulated facts allow only two possibilities for the oxygen pattern, **4** and **5**.



4 **5**

(10) G. M. Badger, R. S. Pierce, and R. Pettit, *J. Chem. Soc.*, 1112 (1952); R. N. Jones, *J. Am. Chem. Soc.*, **62**, 149 (1940); J. W. Cook and A. M. Robinson, *J. Chem. Soc.*, 506 (1938).

In addition to the oxidative results, there are several other grounds for preferring formula **5** to **4**.

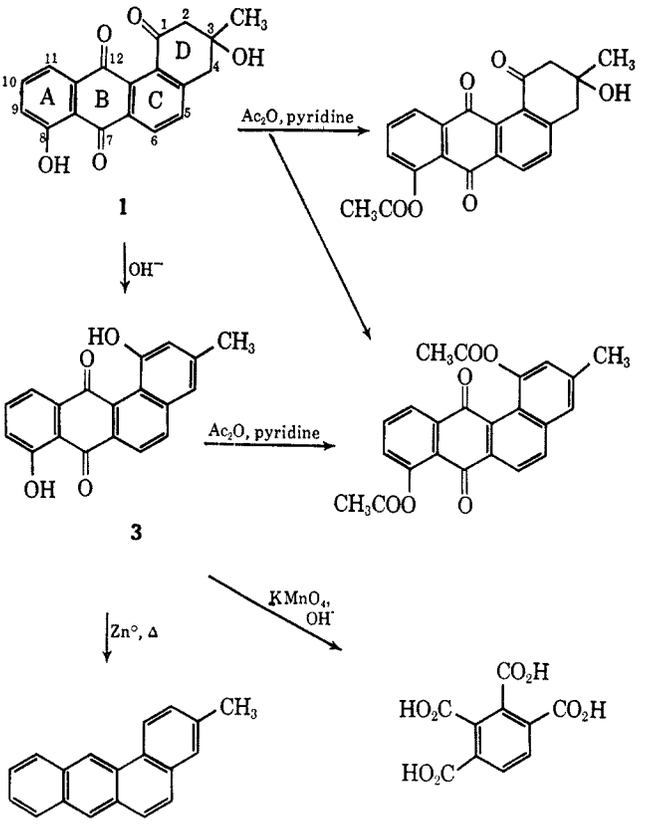
It has been established that polyhydroxynaphthacenequinones absorb light in the visible region at virtually the same wavelength as their anthraquinone counterparts, although at a higher specific absorbancy value.[9] In other words, fusing an additional aromatic ring in a linear fashion to a hydroxyanthraquinone has very little effect on the position of the visible maximum. This finding facilitates the selection of spectral models. Thus it is tempting to extend this concept to the angular benz[*a*]anthraquinone series, and, in fact, the visible maximum of synthetic 8,11-dimethoxybenz[*a*]anthraquinone is similar to that for 1,4-dimethoxyanthraquinone. As the visible spectrum of dimethoxytetrangulol is quite different from 8,11-dimethoxybenz[*a*]anthraquinone, however, formula **4** appears unsatisfactory. Unfortunately a suitable model for the oxygenation pattern represented in formula **5** is presently unavailable. The use of analogy, however, must be applied with caution, for the visible spectra of 1,5-dihydroxy and 1,5-dimethoxyanthraquinone are very similar to those of the corresponding tetrangulol derivatives whereas the degradative and nmr data (see below) clearly rule out position 5 as a site for a hydroxyl. One must always apply analogy with extreme care unless the comparison is a very close one.

The nmr spectrum of dimethoxytetrangulol is clearly unsatisfactory for a derivative of formula **4** regardless of where the methyl group is placed. In any possible combination, a singlet (intensity 1 if the methyl is placed in ring A, or intensity 2 if the methyl group is placed anywhere else in the molecule) is required for ring A hydrogen by formula **4** and none is found. On the other hand formula **5** is entirely consistent with all of the data presented so far and, furthermore, leads to formula **1** as the unique expression for tetrangomycin.

Formula **3** (for tetrangulol) has hydroxyl functions situated so as to chelate with both quinone carbonyls and to labilize all rings except C to alkaline permanganate oxidation. Structural increment **2** represents rings A and B, the *ortho*-situated AB pair in the nmr spectra is due to ring C, and the aromatic methyl group and the two *meta*-coupled protons are present in ring D (see Scheme I).

Formula **3** leads as a natural course to formula **1** for tetrangomycin. The optical activity of the antibiotic is due to the asymmetric carbon in ring D. The portion of the AB quartet in ring C which is due to the hydrogen on C-5 would undergo a downfield shift upon aromatization of ring D. The ease of alkaline aromatization of tetrangomycin is explained by the situation of the nonacetylatable hydroxyl β to the carbonyl group in ring D. The keto function at C-1 becomes the new chelating hydroxyl of tetrangulol upon enolization. The intermediate frequency (1705 cm^{-1}) for the C-1 carbonyl in the infrared spectrum probably is caused by steric and electrostatic repulsive forces between the crowded ketone and the quinone carbonyl, forcing the ketone out of plane. This noncoplanarity would result in decreased π-orbital overlap and a resulting frequency intermediate between an aliphatic-aromatic ketone and a purely aliphatic ketone.[11] Models do indicate, however, sufficient flexibility for ring D to explain the lack of geminal coupling between the hydrogens on the insulated methylene carbons.

SCHEME I



The mass spectra of tetrangomycin and dimethoxytetrangulol, aside from establishing unequivocal molecular formulas, were disappointingly uninformative. After an instructive loss of acetone from ring D of tetrangomycin and a somewhat more mysterious loss of OH from dimethoxytetrangulol, the major degradative pathway in either case involved successive losses of CO from the various carbonyls and phenolic hydroxyls. As all the rings, except C, were so involved, it was not possible to find intact major fragments representing significant portions of those molecules.

On balance, the structural characterization of these two microbial metabolites serves to emphasize the impressive power of modern physical methods when applied to cases of this type. Their isolation represents the first recorded instance of the formation of benz[*a*]anthracene derivatives by living systems and the first report of this unusual hydroxylation pattern within the class. Considering the available methods of synthesis for benz[*a*]anthraquinones, tetrangulol represents an interesting challenge. Despite the chemical novelty of the oxygenation pattern, both structures **1** and **3** fit without difficulty into the currently accepted understanding of the biosynthesis of microbial phenols from acetate–malonate. Their close structural relationship suggests that they are likely to be sequential products along a common pathway although it is not possible to say at the present time which is parent and which is progeny. Although one intuitively favors biological dehydration of tetrangomycin to tetrangulol, biological hydroxylation to interrupt aromatic systems is also a well-known reaction. Tetrangulol is probably *not* an

(11) A similar argument is used for the poor overlap of a carbonyl group with the aromatic nucleus in cordeauxiaquinone: J. H. Lister, C. H. Eugster, and P. Karrer, *Helv. Chim. Acta*, **38**, 215 (1954).

artifact of the isolation procedure for both metabolites are found in the culture filtrates even when special care is exercised to avoid alkaline conditions throughout the process.

Experimental Section

Isolation of Tetrangomycin and Tetrangulol.—*Streptomyces rimosus* (NRRL Culture No. 3016), isolated in our soil screening program, was grown in 300 l. of a sterile culture medium containing 1500 g of Difco peptone, 3000 g of glucose, 6000 g of molasses, and 300 g of calcium carbonate. After 60 to 70 hr of incubation at 28°, using normal aeration and agitation conditions, antibiotic potency reached a maximum. The harvested mash was filtered with the aid of 2% diatomaceous earth and the filtrate was extracted with 200 l. of ethyl acetate. The extract was concentrated to a residue under reduced pressure and inert materials were removed in part by successive treatments with methylene chloride and methanol, in which solvents the metabolites are soluble but some of the impurities are not. Additional inert material, largely oils, was removed by treatment with hexane. The hexane-insoluble material was dissolved in methylene chloride and chromatographed on 300 g of silica gel, taking 500-ml fractions. A colored band eluted in fraction 2. Evaporation under reduced pressure and treatment with ethyl acetate produced purple needles of tetrangulol. Crystallization to constant melting point gave 5 mg of analytically pure material, mp 198–200°. In a subsequent 3000-l. fermentation, 2.41 g of pure tetrangulol was obtained. The ultraviolet spectrum (methanol) shows peaks at 425, 315, sh 250, and 225 mμ (log ε 3.85, 4.39, 4.31, and 4.70) shifting to 465, 380, 325 infl, 290, 230 mμ (log ε 4.06, 3.97, 4.50, 4.26, and 4.85) upon the addition of base.

Anal. Calcd for $C_{19}H_{12}O_4$ (304.3): C, 74.99; H, 3.97; C-methyl, 4.93. Found: C, 74.99; H, 4.03; C-methyl, 3.62; mol wt, 312 (thermistor, chloroform).

The eluting solvent was changed to ethyl acetate after 8 fractions had been taken, and a series of 17 antibacterially active fractions were obtained. Evaporation of the combined fractions gave 6.4 g of a dark oil. The oil was chromatographed on a partition column containing 700 g of acid-washed diatomaceous earth using a solvent system composed of cyclohexane–ethyl acetate–methanol–water (432:216:50:27). The yellow, biologically active, fractions found in the second hold-back volume were evaporated and warmed with methanol. After filtration, concentration to a small volume, addition of ethyl alcohol, and further concentration, 363 mg of tetrangomycin was obtained as bright yellow crystals, mp 182–184°, $[\alpha]^{25}D$ 41.8 ➡ 3.5° (*c* 0.861, $CHCl_3$). The ultraviolet absorption spectrum, in acidic or neutral methanol, showed maxima at 206, 267, 330 (sh), and 400 mμ (log ε 4.43, 4.50, 3.46, and 3.72, respectively).

Anal. Calcd for $C_{19}H_{14}O_5$ (322.3): C, 70.80; H, 4.38; O, 24.82; C-methyl, 4.66. Found: C, 70.84; H, 4.77; O, 24.11; C-methyl, 3.29; mol wt, 322 ± 0 (mass spectrum).

Conversion of Tetrangomycin to Tetrangulol with Base.—A sample of tetrangomycin (10 mg) was warmed with 5 ml of 1 *N* NaOH. The solution immediately turned dark wine red and then a purplish glass precipitated. This was filtered and the precipitate redissolved in a small amount of dimethylformamide. To this was added 1 drop of 1 *N* HCl whereupon purple-bronze needles began to form. Water was added to crystallize nearly all the material and this was filtered to give 7.5 mg of needles, mp 199–201°. This material was identical in its infrared and ultraviolet spectra with an authentic sample of tetrangulol (mp 198–200°) isolated from culture filtrates. A mixture melting point was undepressed (198–200°).

Tetrangulol Diacetate.—A mixture of 0.200 g of tetrangulol and 40 mg of anhydrous sodium acetate and 8.0 ml of acetic anhydride was warmed for 1 hr on a steam bath and then allowed to stand at room temperature overnight. The brownish reaction mixture was poured on ice and the resulting precipitate was removed by filtration and washed with water. The residue was crystallized from acetone–water to give 0.195 g of greenish brown needles, mp 177–178° with solidification and remelting at 186–187°. The infrared spectrum had significant bands at 1770 and 1670 cm^{-1}. The ultraviolet spectrum showed maxima at sh 400, sh 360, sh 338, 290, 242, and 220 mμ (log ε 3.54, 3.67, 3.74, 4.58, 4.43, and 4.65).

Anal. Calcd for $C_{23}H_{16}O_6$ (388.4): C, 71.13; H, 4.15. Found: C, 70.90; H, 4.28.

Acetylation of Tetrangomycin.—A sample of tetrangomycin (~40 mg) was dissolved in acetic anhydride (2 ml) and 1 drop of pyridine was added. The resulting solution was heated on the steam bath for 1 hr. This was cooled and poured into ice water whereupon crystals slowly formed. These were filtered and then recrystallized from acetone–water to give 41 mg of pale yellow needles, mp 148–155°. By thin layer chromatography (Eastman Chromagram, Type K 301R, developed with ethyl acetate) this crystalline material was a mixture. Consequently, the material was dissolved in CH_2Cl_2 and chromatographed on silica gel (8 g). Two main colored bands were eluted from the column: a yellow band with CH_2Cl_2 and an orange band with ethyl acetate.

The yellow band, upon evaporation of solvent and recrystallization from acetone–water, gave 9 mg of pale yellow-brown needles, mp 179–183°. Recrystallization of a small sample from acetone–water gave pale yellow needles, mp 188–189°. These were identical with an authentic sample of tetrangulol diacetate (mp 189–190°) in the infrared and ultraviolet spectra and by thin layer chromatography. A mixture melting point (188–190°) was undepressed.

The orange band, upon evaporation of solvent and recrystallization from acetone–water, gave 22 mg of light yellow needles, mp 181–183°. Recrystallization from acetone–water followed by drying under reduced pressure [100° (10^{-3} mm)] for 1 day gave needles, mp 187–189°, $[\alpha]^{25}D$ 76 ± 3° (*c* 0.988, $CHCl_3$). The ultraviolet spectrum, when measured in neutral or acidic methanol solutions, had maxima at 205, 250 (sh), 264, 334, and 400 (sh) mμ (log ε 4.49, 4.39, 4.52, 3.64, and 2.85, respectively). The ultraviolet spectrum measured in basic methanol had maxima at 226, 300, 325 (sh), 385, and 460 mμ (log ε 4.61, 4.22, 4.12, 3.67, and 3.69, respectively). The infrared spectrum contained carbonyl bands at 1770, 1705, 1670, and 1635 (w) cm^{-1}.

Anal. Calcd for $C_{21}H_{16}O_6$ (364.4)·0.5H_2O: C, 67.55; H, 4.59; O-acetyl, 11.53; C-methyl, 8.05. Found: C, 67.65; H, 4.66; O-acetyl, 12.81; C-methyl, 6.52.

Tetrangulol Tetraacetate.—A mixture of 0.200 g of tetrangulol, 0.500 g of zinc dust, 0.040 g of anhydrous sodium acetate, and 8.0 ml of acetic anhydride was heated under reflux for 0.5 hr. After cooling, the mixture was filtered, poured on ice, and allowed to stand overnight. The resulting oil was extracted into chloroform, which was dried over sodium sulfate and evaporated, to produce 0.406 g of a thick oil. The oil was chromatographed over 5 g of acid-washed alumina in benzene to give 0.236 g of a yellow foam. This was crystallized from benzene–petroleum ether to give 0.19 g of yellow needles. The infrared spectrum showed maxima at 1770 (s) and 1620 (w) cm^{-1}.

Anal. Calcd for $C_{27}H_{22}O_8$: O-acetyl (4), 36.28. Found: O-acetyl, 32.72.

Tetrangulol Mono- and Di-O-methyl Ethers.—A solution of 0.68 g of tetrangulol in 20 ml of hot 20% sodium hydroxide solution (methanol–water, 1:1) was treated with 10 ml of dimethyl sulfate. The hot solution was treated with the following sequence of reagents: 20 ml of 25% sodium hydroxide, 20 ml of dimethyl sulfate, 20 ml of 25% sodium hydroxide, and finally 10 ml of dimethyl sulfate. The rates of addition were adjusted so as to maintain incipient boiling throughout. The reaction mixture was then extracted with chloroform, the chloroform extracts were dried with anhydrous sodium sulfate, concentrated to a small volume and passed over 100 g of neutral alumina. The dimethyl ether, mp 190–191°, eluted with chloroform and was crystallized from benzene–hexane to give 0.25 g of yellow needles. The ultraviolet spectrum in ethanol showed peaks at 380, 350, 302, and 221 mμ (log ε 3.78, 3.79, 4.48, and 4.67).

Anal. Calcd for $C_{21}H_{16}O_4$ (332.3): C, 75.89; H, 4.85; O-methyl (2), 9.02. Found: C, 75.53; H, 5.15; O-methyl, 8.71; mol wt, 332 ± 0 (mass spectroscopy).

Chloroform containing 10% methanol eluted the monomethyl ether, mp 202–203°, which crystallized from benzene–hexane to give 0.09 g of fire-red needles.

Anal. Calcd for $C_{20}H_{14}O_4$·0.25H_2O (322): C, 74.45; H, 4.78; O-methyl, 4.64. Found: C, 74.59; H, 4.58; O-methyl, 4.60.

Zinc Dust Distillation of Tetrangulol.—An intimate mixture of 0.160 g of tetrangulol and 3.75 g of zinc dust was divided into eight portions and heated with a bunsen burner in knee tubes. The tubes were broken at their bends and the sublimates were dissolved in chloroform and combined. The solvent was carefully removed and the residue was chromatographed on 2 g of alumina using petroleum ether. The principal product eluted with benzene and was purified by sublimation followed by two

crystallizations from methylene chloride–methanol to give a very small amount of colorless plates, mp 146–148°. A qualitative ultraviolet spectrum showed the following maxima in ethanol: 384.5, sh 374, 358.5, 342, 328, 314, 300, 287, 277, and 268 mμ.

Alkaline Permanganate Oxidation of Tetrangulol.—Tetrangulol (0.37 g) was suspended in 100 ml of 1 *N* potassium hydroxide solution and heated to dissolve as much as possible. Potassium permanganate solid (7.5 g) was added in small portions while heating on the steam bath, and after an hour of additional heating, the suspension was allowed to stand at room temperature overnight. The excess permanganate was destroyed with methanol and the suspension was filtered and washed with 1 *N* sodium hydroxide solution. The water-clear filtrate was strongly acidified with hydrochloric acid and concentrated to about 25 ml. After cooling, the precipitated salts were filtered, and the filtrate was extracted with four 25-ml portions of ethyl acetate. After drying over anhydrous sodium sulfate, filtration, and evaporation, 0.208 g of a white powder was obtained which was dissolved in 5 ml of concentrated nitric acid and heated for 45 min on a hot plate. When the nearly clear solution had concentrated to about 1 ml, the flask was cooled and 55 mg of a crystalline precipitate separated; after two recrystallizations from concentrated HNO_3, the benzene-1,2,3,4-tetracarboxylic acid melted constantly at 233–236° (lit.[12] mp 236–238°). A 20-mg sample of the acid was esterified in the usual fashion with ethereal diazomethane generated from 300 mg of N-methyl-N′-nitro-N-nitrosoguanidine. After 5 min, the excess reagent was destroyed with dilute, aqueous acetic acid, and the ethereal solution was washed with 1 *N* base, followed by water, dried over anhydrous sodium sulfate, and was evaporated. The residue was purified by partition chromatography on 5 g of acid-washed diatomaceous earth using a system composed of hexane–ethyl acetate–methanol–water (80:20:16:6). The sixth and seventh hold-back volumes contained virtually all of the solid and these gave, after several crystallizations from methanol, needles of methyl benzene-1,2,-3,4-tetracarboxylate melting constantly at 128–129° (lit.[13] mp 130–131°). The well-known[13] formation of a purple color upon exposure of the crystals to light was observed with our sample as well.

As anticipated, sublimation of the acid produced an anhydride (infrared bands at 1800–1860 cm^{-1}) whose melting point behavior was complex and nonreproducible.

Synthesis of 8,11-Dimethoxybenz[*a*]anthraquinone.—A mixture of 460 mg of 3,6-dimethoxyphthalic anhydride (prepared by hydroysis of 3,6-dimethoxyphthalimide by an unpublished procedure of Dr. H. S. Corey of these laboratories) and 256 mg of naphthalene in 50 ml of tetrachlorethane was chilled and 534 mg of anhydrous aluminum chloride was added in small increments. After stirring overnight at room temperature, the dark suspension was poured onto ice and acidified with sulfuric acid. The solution was extracted into chloroform and the dried chloroform layers were concentrated to produce 211 mg of a dark oil. This gradually crystallized in part to give 46 mg of an acid. The acid was not purified further but was dissolved in 10 ml of 90% sulfuric acid and warmed 5 min on a steam bath. The solution was poured onto ice, the product was obtained by chloroform extraction, and the chloroform layers were washed with 5% sodium hydroxide solution, dried, and evaporated to produce 31 mg of an orange-red residue. The amorphous residue was crystallized from methylene chloride–methanol to give 18 mg of the desired benz[*a*]anthraquinone which did not melt below 325°. The ultraviolet spectrum in methanol showed maxima at 236, 280, and 430 mμ (log ε 4.14, 4.04, and 3.71).

Anal. Calcd for $C_{20}H_{14}O_4 \cdot 0.333H_2O$: C, 74.11; H, 4.55. Found: C, 74.05; H, 4.32.

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Alkaloids of the *Papaveraceae*. IV. *Argemone hispida* Gray and *A. munita* Dur. & Hilg. subsp. *rotundata* (Rydb.) G. B. Ownb.[1]

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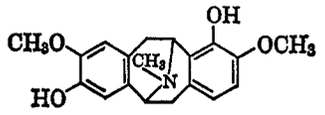
The alkaloid contents of *Argemone hispida* Gray and *A. munita* Dur. & Hilg. subsp. *rotundata* (Rydb.) G. B. Ownb. have been established and compared with other *Argemone* species in a search for biogenetic and chemotaxonomic relationships. A new alkaloid, munitagine, has been isolated from *A. munita* and its structure has been shown to be 2,7-dihydroxy-3,8-dimethoxy-5,6,11,12-tetrahydrodibenzo[*a*,*e*]cyclooctene-N-methyl-6,12-imine. This represents the first alkaloid of the dibenzocyclooctene group to exhibit an altered substituent pattern.



The structures of all alkaloids of this group, including munitagine, can be correlated with that of reticuline, which was found to occur in both species.

The discovery and structure elucidation[1,4] of the interesting argemonine (5,6,11,12-tetrahydrodibenzo-[*a*,*e*]cyclooctene-N-methyl-6,12-imine) alkaloids (I) have provided new impetus for further investigations in some of the rarer genera of the poppy family. Since, with one exception,[4d] alkaloids of structure I have only been found in *Argemone* species, this genus seemed to provide the most fruitful place in which to search for new examples and to attempt to uncover biogenetic relationships. In addition, a recent excellent botanical monograph[5] critically reevaluates plant classifications and provides the comparison basis for chemotaxonomy

(1) Part III: F. R. Stermitz and J. N. Seiber, *Tetrahedron Letters*, in press. The present study was presented in part at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965.

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