THE TOTAL SYNTHESIS OF A TETRACYCLINE*

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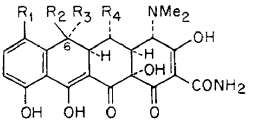
The tetracyclines are substances built upon the pattern of a unique highly oxygenated hydronaphthacene structure (*Figure 1*). They are produced in the course of the metabolism of various *Streptomyces* species, or by simple chemical modification of naturally-occurring members of the class. Aureomycin, or chlorotetracycline, was the first of these unusual substances to be



	R_1	R_2	R_3	R_4
CHLOROTETRACYCLINE (AUREOMYCIN)	Cl	OH	Me	H
OXYTETRACYCLINE (TERRAMYCIN)	H	OH	Me	OH
TETRACYCLINE	H	OH	Me	H
6-DIMETHYL-6-DEOXYTETRACYCLINE	H	H	H	H

Figure 1

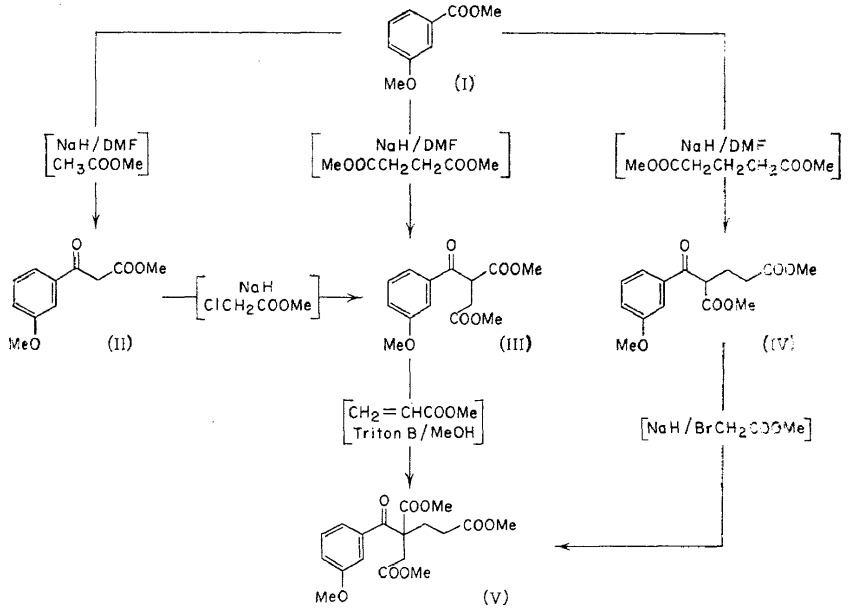
discovered, and its pronounced activity against a broad spectrum of pathogenic organisms rapidly gained for it an outstanding place in medical practice. Shortly thereafter, oxytetracycline, or Terramycin, assumed a similar rôle, as, later still, did tetracycline and 6-demethyltetracycline. Tetracycline can be obtained directly from natural sources, or from Aureomycin by removal of chlorine, while 6-demethyltetracycline is, so far, strictly a natural product. From the latter, 6-demethyl-6-deoxytetracycline, the prototype and simplest member of the entire group, can be obtained by removal of an hydroxyl group. The characteristic chemotherapeutic activity of all of these substances is essentially equivalent, and is strictly dependent upon the maintenance of all of the structural and stereochemical features of the general structure shown in *Figure 1*. That is to say, while the groups R_1 through R_4, situated along the upper periphery of the molecule, can be varied over a considerable range without effecting a substantial change in antibiotic properties, any modification elsewhere in the array leads to a marked decrease or complete loss of biological activity.

The structures of Terramycin and Aureomycin were elucidated in our Laboratories a decade ago. Since that time, the complicated tetracyclic

*A brief communication recording the results on which this lecture is based has appeared in *J. Am. Chem. Soc.* **84**, 3222 (1962).

assemblage, adorned with an unusual number of contiguous reactive functional groups of different kinds, and replete with stereochemical imperatives, has presented a synthetic challenge to which many have responded. Outstanding progress towards the objective has been achieved in several laboratories; special mention may be made of the contributions of Muxfeldt in Germany, Shemyakin in Russia, and Fields, Kende and Boothe in the United States. But until now, no member of the tetracycline group has been reached by synthesis. It is my purpose here to describe the achievement of that goal with the total synthesis of 6-demethyl-6-deoxytetracycline.

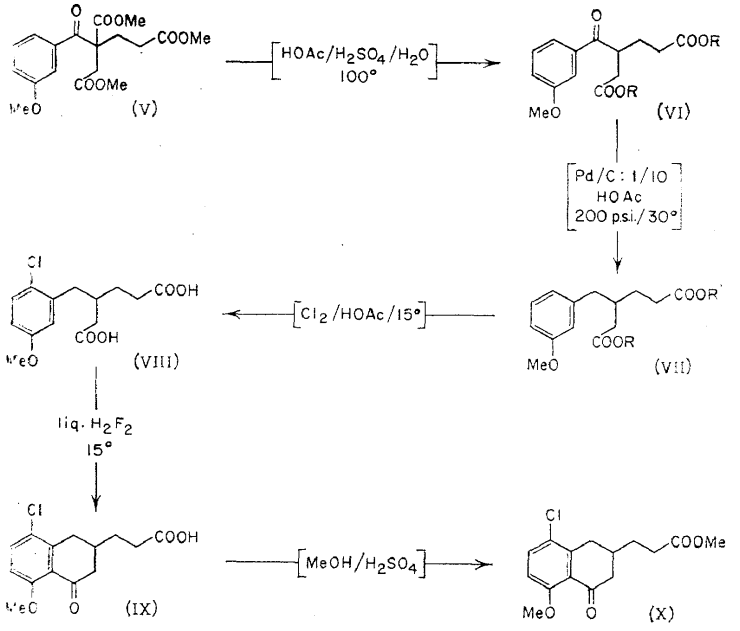
It was our plan to use the aromatic ring present in the molecules of the tetracyclines as a foundation stone, on which the further, more complicated rings might be elaborated. Our first operations were very simple ones, designed for the obtention of dimethyl β-carbomethoxy-β-(*m*-methoxybenzoyl)adipate (V), which was in fact found to be readily preparable by either of three methods. In each case, our starting material was methyl *m*-methoxybenzoate (I), which, in dimethylformamide solution, in the presence of sodium hydride, was condensed with either methyl acetate, dimethyl succinate, or dimethyl glutarate. In the first of these methods, which was found in practice to be the best, the resulting methyl *m*-methoxybenzoylacetate (II) was alkylated directly, without isolation from the original condensation mixture, by treatment with methyl chloroacetate, to give dimethyl *m*-methoxybenzoylsuccinate (III). Alternatively, the latter was obtained



directly by the dimethyl succinate condensation. In either event, the keto-diester (III) participated in a smooth Michael condensation with methyl acrylate, which was carried out in dioxan solution, with methanolic Triton B as catalyst, and gave the desired keto-triester (V). In the third, but rather

less satisfactory, method the same intermediate was prepared from (IV), the product of an initial dimethyl glutarate condensation, by alkylation with methyl bromoacetate.

The keto-triester (V) was now subjected to the action of hot aqueous acetic and sulphuric acids, which brought about hydrolysis and decarboxylation in the normal fashion, and yielded β-(*m*-methoxybenzoyl)adipic acid (VI: R = H). It may be noted parenthetically here that the ketonic carbonyl group of this acid is so sited as to correspond to the 6-position of an eventual tetracycline, and that the nature of the grouping is such as to permit, in principle, the incorporation, at this point, of any of a variety of substituents. For present purposes, we are interested only in the simplest of the possible constructions, which was brought about through hydrogenation of the keto-acid, in acetic acid, under pressure, in the presence of palladium/charcoal, to give β-(*m*-methoxybenzyl)adipic acid (VII: R = R′ = H). In practical operations carried out on a large scale, it was found to be



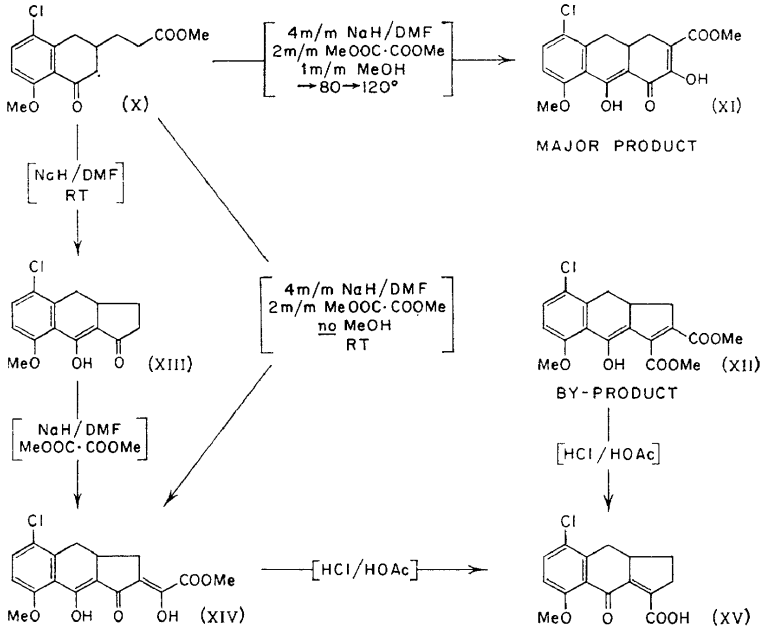
felicitous to make certain simple modifications in this scheme. Thus, the acid VI (R = H), from hydrolysis and decarboxylation, was not isolated in the pure state, but was directly converted into the corresponding ester (VI: R = Me), which was purified by distillation, and subjected to hydrogenation. In this case, the product of the reduction—which undoubtedly proceeds through an intermediary γ-lactone—was the half ester VII (R = H, R′ = Me), which was fully esterified, purified by distillation, and hydrolyzed to the acid (VII: R = R′ = H) by aqueous sodium hydroxide.

It was now our aim to complete the construction of the second of the six-membered rings we must build, by an internal cyclization reaction. But the

acid VII (R = R′ = H), as such, was ill-suited to our purpose, since cyclization would certainly in large measure involve the preferred attack of a cationoid centre on the aromatic ring at the position *para* to the methoxyl group, rather than at the *ortho* position, as required. In order to circumvent this difficulty, blocking of the too-eager *para* position was necessary, and was brought about by treatment of the acid (VII: R = R′ = H) with chlorine in acetic acid at 15°, to give β-(2-chloro-5-methoxybenzyl)adipic acid (VIII). The latter was now smoothly cyclized to the desired tetralone (IX) by liquid hydrogen fluoride. The *ortho* disposition of the methoxyl and carbonyl groups in the new ketone was easily demonstrable through spectroscopic observations, but it was necessary at this point to allay some concern about a further structural point. Although it seemed highly probable that our cyclization product was the six-membered ring ketone (IX), the possibility could not *a priori* be completely excluded that the cyclization had involved the second of the available carboxyl groups in (VIII), and led to the construction of a new *seven*-membered ring. The availability of the monoester VII (R = H, R′ = Me) enabled us to show that such was not the case, since that substance, subjected to a parallel sequence of chlorination and cyclization reactions, gave the same product as that obtained from the diacid VII (R = R′ = H). Now, with the conversion of the acid (IX) into the corresponding methyl ester (X), the first phase of our work was complete, and the stage was set for the elaboration of yet another ring. The practical aspect of the work so far described may be summarized with the observation that the tetralone acid (IX) was preparable on the kilogram scale, without extensive developmental work, in 10 per cent over-all yield from methyl *m*-methoxybenzoate (I) (170 grams [IX]/1000 grams (I)].

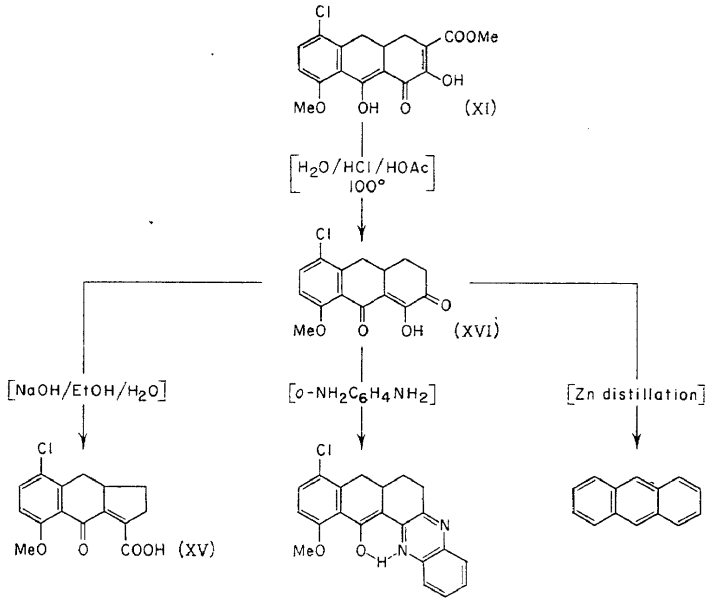
We had it in mind to effect the construction of a third six-membered carbocyclic ring by condensation of the tetralone ester (X) with methyl oxalate, to give the tricyclic substance (XI), and that objective was in fact satisfactorily achieved, but not without extensive experimentation. It will be noted that the ester (X) is so constituted as to be susceptible to *internal* condensation, and indeed, it is transformed almost quantitatively into the simple tricyclic compound (XIII), under the influence of sodium hydride, in dimethylformamide at room temperature. It need occasion no surprise, therefore, that this same internal cyclization plays a definitive rôle, under certain conditions, in condensations involving methyl oxalate. Thus, when the latter and the ester (X) were condensed in dimethylformamide, in the presence of sodium hydride, at room temperature *in the absence of added methanol*, the product (XIV) was obtained in 50 per cent yield; this same substance could be obtained from (XIII) by reaction with methyl oxalate, under similar conditions, in 80 per cent yield. Its structure was clear from its spectroscopic properties, and its hydrolysis by hot acetic and hydrochloric acids to the acid (XV), a process which clearly involves cleavage of the five-membered ring of (XIV), followed by decarboxylation, and cyclization of the resulting open-chain intermediate in an alternative manner. By contrast, when the sodium hydride-catalysed condensation of the ester (X) and methyl oxalate was carried out at an elevated temperature, in the presence of a limited amount of methanol, the desired hydroanthracene ester (XI) was obtained in 45 per cent yield. As yet, we have been unable to

bring about this reaction without the concomitant formation, in 20 per cent yield, of the by-product (XII). This substance, like (XIV), but clearly by a simpler series of changes, is transformed to the keto-acid (XV) by treatment with hot aqueous hydrochloric and acetic acids. The formation of (XII) presents no mechanistic problem, except perhaps in matters of detail, but it

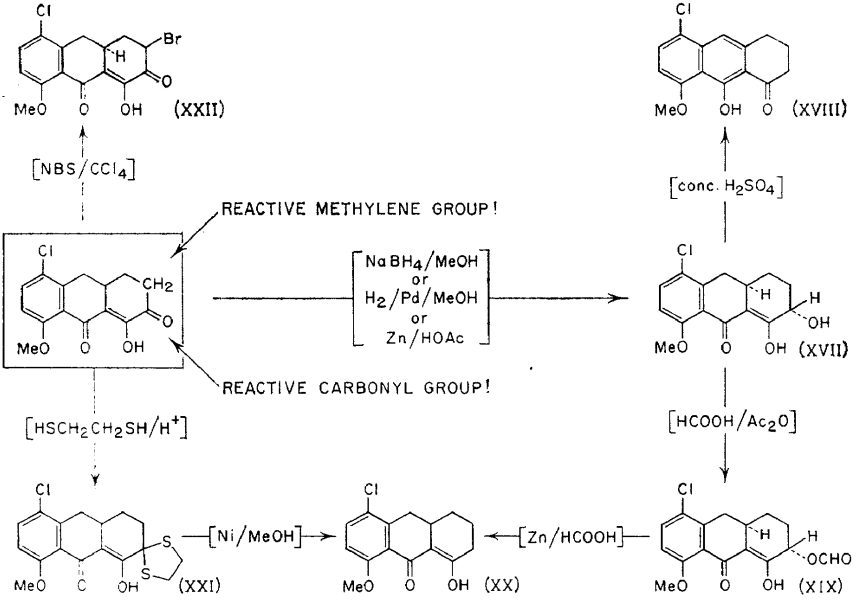


is less clear how the changes which lead to the by-product might be suppressed. Special note may be made here of the capacity of the sodium hydride/dimethylformamide combination to bring about condensations which might be expected to be especially difficult, particularly as demonstrated in the reactions leading to (XII) and (XIV). We were to find opportunity to make favourable use of this capacity at a later crucial stage of our work.

When the tricylic ester (XI) was subjected to hydrolysis, again with hot hydrochloric and acetic acids, it was smoothly transformed, in 75 per cent yield, into the hydroanthracene ketone (XVI). This substance is a key intermediate in our investigations, and we felt it desirable to establish its structure beyond any possible question. All relevant physical characteristics of the material presented no conflict with the assigned structure, but in the absence of exact models, the data could not be regarded as conclusive. On the other hand, zinc dust distillation of the ketone gave anthracene in high yield, and reaction with *o*-phenylenediamine led to a quinoxaline, whose properties left no doubt of the presence in its molecule of a strong hydrogen bond. Finally, (XVI) was transformed, by easily formulable changes, into the now familiar keto-acid (XV), in this case by the action of aqueous ethanolic sodium hydroxide.

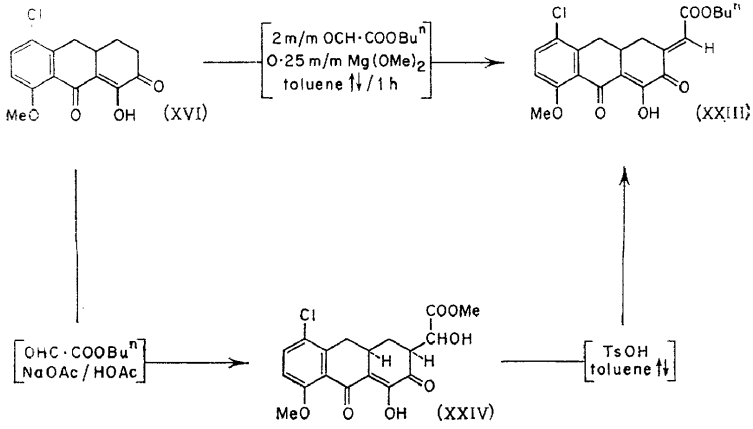


We anticipated that the key tricyclic ketone (XVI) (see box, in diagram on p. 567) would exhibit special reactivity in two directions, which would make it an especially favourable and flexible vehicle for the fusion of a fourth and final ring, according to one of a number of possible designs. Thus, it seemed likely that two of its three carbonyl groups would co-operate in the formation of a stabilized vinylogous carboxylic acid system, while the third, like that in simple α-keto-acids, should be both highly susceptible to addition reactions, and readily enolizable. This latter circumstance, we felt, should convey high reactivity on the adjacent methylene group, and permit ready condensations at that centre. These presumptions found ample justification in our preliminary examination of the reactions of (XVI). Thus, the ketone was readily convertible into a mono-thioketal (XXI), and as readily reduced, catalytically or by chemical combinations, to the dihydro-derivative (XVII). From the latter, the alcoholic hydroxyl group could be easily removed by formylation and reduction with zinc and formic acid—a series of changes which established a precedent for an important subsequent sequence. The transformation of (XVII) by sulphuric acid into the naphthalenoid compound (XVIII) is of special (though here parenthetical) interest in the pattern it might foreshadow for the synthesis of substances of the anhydrotetracycline class. The relative chemical stability of the enolized β-dicarbonyl system was further evidenced by the smooth Raney nickel desulphurization of the thioketal (XXI), which provided an alternative route to the deoxo compound (XX). The occurrence of all of these reactions in the fashion shown was very readily established through the observation, in the products (XVII), (XIX), (XX) and (XXI), of highly characteristic infra-red and ultra-violet spectra, for which excellent models were available. Finally,



the α-methylenic reactivity of the ketone (XVI) was strikingly apparent in its transformation in high yield to a monobromo-derivative, (XXII), when it was treated with *N*-bromosuccinimide in carbon tetrachloride solution.

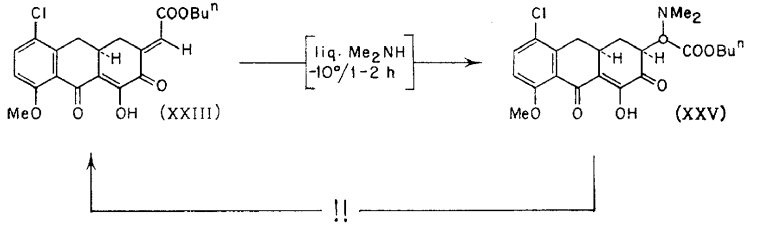
We may now return to the main path of our synthetic progression. The ketone (XVI), manifesting the anticipated α-methylenic reactivity, combined with n-butyl glyoxylate, in a reaction carried out in boiling toluene and catalysed by magnesium methoxide, to give the glyoxylidene derivative (XXIII) in 60 per cent yield. Under milder conditions, the same components



could be brought together to give the hydroxyester (XXIV), which in its turn was dehydrated to (XXIII) when it was heated in toluene in the presence of *p*-toluenesulphonic acid. But no advantage attached to the

longer route. It is noteworthy that only one isomer of (XXIII)—presumably the *trans* compound, as shown—was isolated from these reactions.

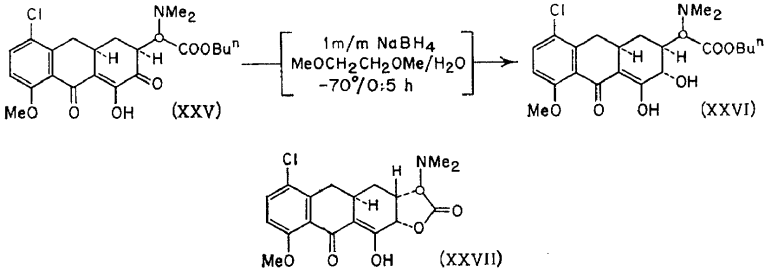
We had hoped to, and did indeed, find in the intermediate (XXIII) the high susceptibility to addition of nucleophiles which is a usual characteristic of substances containing an olefinic bond flanked by electron-withdrawing



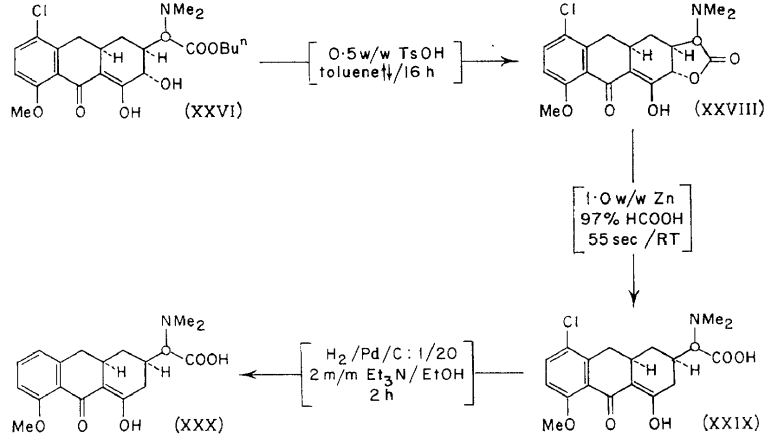
substituents. When (XXIII) was dissolved in liquid dimethylamine, addition took place with great facility to give the desired base (XXV). But the reaction was extraordinarily readily reversible; when the excess dimethylamine was removed from such reaction mixtures, complete reversion occurred, and the residue consisted of pure (XXIII). Under properly chosen conditions, the addition product could be isolated by bringing about its crystallization directly from the reaction mixture. In this way, it was adequately characterized by physical measurements, but was far too unstable to permit the obtention of satisfactory elementary analytical figures. Needless to say, these circumstances complicated the manipulation of (XXV) in a forward direction, but they also had their bright side. Until now, all of our intermediates had contained only a single asymmetric carbon atom, and it had not been necessary to pay any heed to stereochemical questions. Now, with the formation of (XXV), two new asymmetric centres had been introduced—both of them destined to appear in the final tetracycline. Fortunately, that same ready reversibility which complicated our operations at this point also assured us that the very bulky substituent attached to the hydroanthracene nucleus must adopt the stable, equatorial disposition. Examination of models left no doubt that the stable arrangement is that shown in (XXV), with the new ring asymmetric centre disposed as required. On the other hand, we did not have—nor have we now—a strong conviction in respect to the arrangement of the groups at the second newly created asymmetric centre in (XXV), and left it unassigned. The matter was not definitive, since we believed that the centre would be susceptible to manipulation in the desired sense at a later stage.

The instability of the addition product (XXV) is of course consequent upon the conjunction of a carbonyl group and a β-situated dimethylamino group, and the practical difficulties posed by the circumstances described above were overcome by carrying out directly a further step in which the elimination-triggering carbonyl group was destroyed. Thus, after the glyoxylidene derivative (XXIII) had been allowed to stand for an hour in dimethylamine solution at $-10°$, the temperature was lowered to $-70°$, and sodium borohydride in moist 1,2-dimethoxyethane was added directly, to reduce

the adduct (XXV) to the stable alcohol (XXVI). From such a reaction-sequence, (XXVI) could be isolated as the pure crystalline hydrochloride in 60 per cent yield, over-all from (XXIII). The stereoselectivity of the changes is marked; although in all *three* asymmetric centres are generated, the only other product which we could isolate, in 5 per cent yield, is a readily separable lactone, probably of the structure (XXVII). The equatorial orientation of the hydroxyl group in (XXVI) is consistent with the relative stability of the hydroxyester *vis-a-vis* lactonization.



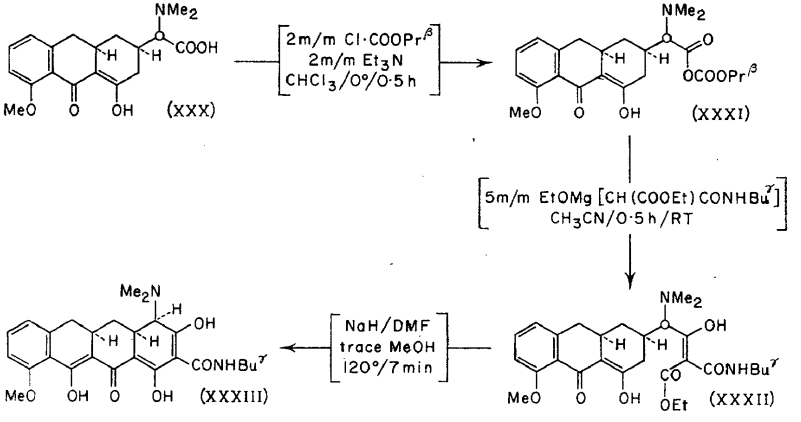
Although lactonization of (XXVI) is not ready, we found that it can be brought about under vigorous conditions. Indeed, the lactone (XXVIII) was produced in essentially quantitative yield when the hydroxyester was heated in boiling toluene for a prolonged period with half its weight of *p*-toluenesulphonic acid. Next, the lactone (XXVIII) was very readily reduced by short treatment with zinc and formic acid, to give the dimethylaminoacid (XXIX) in 85 per cent yield, and the chlorine-free acid (XXX)



was smoothly prepared from the latter in 85 per cent yield by catalytic hydrogenation over palladium/charcoal, in the presence of triethylamine.

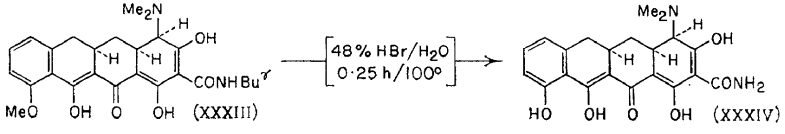
It was now necessary to complete the construction of the fourth and final ring of the tetracycline skeleton, and it seemed probable that the remaining

needed carbon atoms could be attached to the molecule of the acid (XXX) through the agency of its carboxyl group. As a first step, the latter was activated through conversion to the isopropylcarbonic mixed anhydride (XXXI). The pure crystalline anhydride could be prepared in virtually quantitative yield by treatment of the acid (XXX) with isopropyl chloroformate and triethylamine, but there was found to be no advantage in



isolating (XXXI), which was better brought directly into reaction with the ethoxymagnesio derivative of ethyl *N*-t-butylmalonamate, itself prepared from ethyl cyanoacetate and isobutylene in the presence of sulphuric acid. The success of the acylation was dependent on strict adherence to a narrowly defined set of reaction conditions, and the crude product (XXXII) was again best carried along directly into the subsequent cyclization. The latter was brought about—by now, no one will be surprised to learn—by treatment of (XXXII), in dimethylformamide, with sodium hydride, first at room temperature, and then, after addition of a small amount of methanol, for a short time at 120°. In this reaction as in the preceding one, to depart from a highly rigid and laboriously established experimental protocol was to court disaster, but a carefully executed series of operations permitted the consistent preparation of the pure crystalline cyclization product (XXXIII) in 20 per cent over-all yield from the acid (XXX), allowance being made for the recovery of up to 20 per cent of the latter. We cannot but direct attention to the fact that it is less remarkable that the discovery of proper conditions for the cyclization reaction was difficult, than that they could be found at all. For the intermediate (XXXII) contains no less than three very active hydrogen atoms, which must be ionized by sodium hydride; the ensuing cyclization then involves the loss of yet a further proton, at an improbable site, and attack of the resulting anionoid carbon atom on a carbonyl group which has been strongly deactivated through its involvement in one of the initially formed anionoid systems. Among several instances encountered in our work, this final cyclization provides the most dramatic evidence of the efficacy of the sodium hydride/dimethylformamide combination in bringing about even anionoid condensations which must hitherto have been regarded as difficult to the point of improbability.

The next stage in our work represented a welcome relaxation from the strenuous efforts required to master the intricacies attendant upon the construction of our fourth ring, and a useful respite before a further phase of our investigation which would again put our experimental capacity to a severe test. When the pure tetracyclic intermediate (XXXIII) was treated for a short time with hot aqueous hydrobromic acid, the *N*-t-butyl and *O*-methoxyl groups present were both smoothly cleaved, and the dealkylated compound (XXXIV) separated directly from the reaction mixture as the crystalline hydrobromide in almost quantitative yield. Further, since this

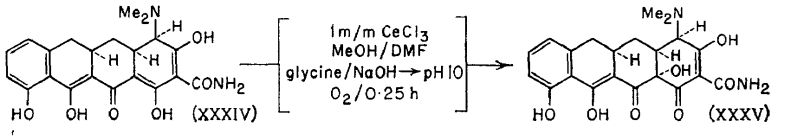


same substance (XXXIV) is readily available in optically active form by deoxygenation and epimerization (at C–4) of 6-demethyl-6-deoxytetracycline, we were able at this point to experience the not inconsiderable satisfaction of establishing, through careful comparative spectroscopic and paper chromatographic studies of samples of (XXXIV), prepared respectively by synthesis and from natural sources, that our synthetic progression had in fact followed the course set forth here.

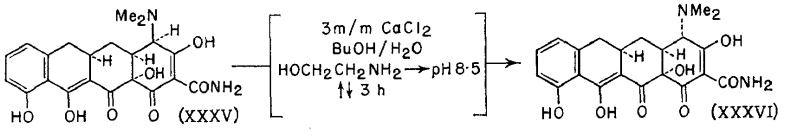
Two points of special interest now deserve comment. It may be regarded as remarkable that the β-polycarbonyl system of (XXXIV) survives the rather vigorous hydrolytic conditions of the hydrobromic acid cleavage reaction, since scission of any one of no less than *seven* contiguous carbon–carbon bonds along the lower and right periphery of the molecule would hardly have presented an occasion for surprise. But we were of course able to establish in advance, through study of (XXXIV) from natural sources, that the possible adverse degradative changes do not in fact occur, at least under conditions of quite sufficient vigour to bring about the desired dealkylations. Finally, it may be noted that the placement of the dimethylamino groups in (XXXIII) and (XXXIV) in the β-orientation depends in part on model arguments which can be developed now, and in part on the interpretation of experiments yet to be described. In the first instance, our examination of models suggests that a β-oriented dimethylamino group in these compounds represents the more stable of the two possible dispositions, since the grouping, though formally *quasi* axially situated, completes in steric demand with only a single *quasi* axial hydrogen atom (at C–5), and is free of the considerable steric compression with which the contiguity of the oxygen atom at C–3 must burden it in the alternative configuration.

It now remained only to introduce an hydroxyl group at C–12a, in the correct stereochemical sense, and invert the dimethylamino group at C–4. Our correlative studies with many substances of the general type (XXXIV), derived from natural tetracyclines, provided a basis for optimism that the desired changes could be brought about, since we had found that each of these analogues, with varying facility from case to case, could be oxygenated in the desired manner by treatment with molecular oxygen in the

presence of a variety of metal ions. In the event, the case at hand was by no means the smoothest we had encountered. Nevertheless, when (XXXIV) was oxygenated for a short period, in carefully buffered methanol/dimethylformamide solution, in the presence of cerous ions, conversion to (XXXV)



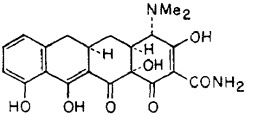
took place, and after the crude reaction mixture had been treated under conditions known to be efficacious in bringing about epimerization of tetracyclines at C–4, pure crystalline totally synthetic racemic 6-demethyl-6-deoxytetracycline (XXXVI) could be isolated in 25 per cent over-all yield.



This easy statement should not be allowed to conceal the formidable effort which was required to bring us to a favourable outcome. The detailed mechanism of the oxygenation reaction is obscure—though there is little doubt that metal chelate formation must play a rôle—and the definition of useful reaction conditions required much experimentation; in view of the number of possibly relevant variables, it may strongly be doubted that the optimum has yet been achieved. Some competitive oxygenation occurs at C–11a, and there is some further transformation of the desired (XXXV); the latter factor requires that the reaction be stopped well before conversion of the starting material (XXXIV) is complete. These circumstances posed a challenging experimental problem, for the solution of which the development of very effective separation procedures, utilizing partition chromatography and countercurrent distribution, was of primary importance.

The care with which it was necessary to examine the reaction mixtures in the last two steps of our synthesis does enable us to conclude with some confidence that the oxygenation reaction does *not* give rise to any compounds oxygenated at C–12a with the unnatural C–12a epi [rings A/B *trans*] configuration. We attribute this favourable steric result to the β-orientation, in (XXXIV), of the dimethylamino group, whose considerable bulk effectively shields the β-face of the trigonal C–12a.

The identity of our synthetic 6-demethyl-6-deoxytetracycline was established beyond question by spectroscopic and paper chromatographic studies, which are summarized in *Figure 2*. It is of particular interest that the synthetic racemic compound is just half as active against pathogenic organisms as its natural counterpart; the not surprising, but none-the-less interesting, conclusion may be drawn that the unnatural isomer is completely devoid of biological activity.



(±) 6-dimethyl-6-deoxytetracycline

		ε	
Ultra-violet spectrum			
in MeOH/0·01 N HCl	267 mμ	19,300	Identical with data for laevorotatory material from natural sources
	347	15,500	
in MeOH/0·01 N NaOH	248	16,600	
	~261	15,600	
	~284	10,100	
	383	18,300	
Paper chromatographic behaviour			
in toluene/pyridine/water			Identical with data for laevorotatory material from natural sources
nitromethane/butanol/toluene/pyridine			
ethylacetate/nitromethane/chloroform			
ethylacetate/chloroform/pyridine			
ethylacetate/water			
Antibiotic activity			
against K. pneumoniae 500 units [tetracycline = 1000]			Exactly ½ activity of laevorotatory material from natural sources

Figure 2

Thus has the first total synthesis of a tetracycline been successfully concluded. Many challenges and opportunities remain. The way is now available for the synthesis of a variety of unique tetracyclines which would be unobtainable from natural sources. Our synthesis is susceptible of improvement and modification in a number of directions. Should practical considerations be paramount, an improved and simpler synthesis of our key tricyclic intermediate (XVI) might well be found, and an especially fascinating problem lies in the design of alternative methods for elaborating the final carbocyclic ring—particularly methods which retain that oxygen atom in (XVI) which might become the C–12a hydroxyl group of a finished tetracycline.

My account of this investigation cannot have done other than emphasize the extent to which the success of our work depended more upon the experimental skill and ceaseless application of my collaborators than upon the virtues of the rather simple plan which was our guide. It is a great pleasure for me here to express my appreciation to these men, and my admiration for their work. Dr Lloyd Conover, with whom it has been my privilege to have been associated for more than eleven years—since my first association with the tetracyclines—shares with me the particular pleasure of having reached a synthetic objective first defined by our own structural work a decade ago, and deserves my special thanks, while Drs Kenneth Butler, J. D. Johnston and J. J. Korst merit the highest praise for their splendid contribution. Finally, all of us cannot but thank with all warmth the management of Chas. Pfizer and Co., Inc., in whose Medical Research Laboratories at Groton this investigation was carried out, for having afforded us a most unusual scientific opportunity.