temperature with 15 ml. of methyl iodide. The excess methyl iodide was then removed and the aqueous solution shaken twice more with 15-ml. portions of methyl iodide each time. The combined methyl iodide extracts were washed once with water and the excess methyl iodide evaporated on the steam-bath to give 1.5 g. of a light green product, m.p. 165–167°. Recrystallization of this product from a small volume of water raised the m.p. to 171–172°.

Anal. Calcd. for $C_7H_8N_4S$: C, 46.6; H, 4.5. Found: C, 46.6; H, 4.7.

This product when mixed with XI prepared by method (1) gave a mixed m.p. of 171–172°. Identical ultraviolet absorption spectra were obtained for XI prepared by method (1) and method (2).

Preparation of 9-Methyl-6-*p*-bromophenoxypurine.—To 5.0 g. of *p*-bromophenol and 5.0 g. of potassium hydroxide dissolved in 150 ml. of water was added slowly 5.0 g. of 9-methyl-6-chloropurine (VII). The solution was heated on the steam-bath for 0.5 hr., cooled and filtered. The crude product[2] was recrystallized from a mixture of ethanol and water to yield 6.1 g., m.p. 164–165°.

Anal. Calcd. for $C_{12}H_9N_4OBr$: C, 47.2; H, 3.0; N, 18.3. Found: C, 47.2; H, 3.0; N, 18.7.

The ultraviolet absorption spectra in absolute ethanol showed a maximum at 256 mμ, ε 18,300.

Preparation of the 9-Methyl-6-substituted-aminopurines Listed in Table I. Method A.—Five grams of 9-methyl-6-chloropurine (VII) was dissolved in 150 ml. of methanol. To this solution was added the 10 to 15 ml. of the amine or aqueous solution of the amine. The solution was heated for 1 hr. on the steam-bath, and the solution (volume approximately 40 ml.) was cooled and filtered. The crude product was recrystallized from the solvents indicated.

Method B.—To 150 ml. of ethanol was added 20 ml. of the appropriate amine or 20–30 ml. of an aqueous solution of the amine. Five grams of 9-methyl-6-chloropurine (VII) was then carefully added and the solution evaporated to dryness on the steam-bath. The solid residue was extracted with three 100-ml. portions of boiling benzene. The benzene was concentrated and cooled and the desired product allowed to crystallize. The product was purified by recrystallization from the appropriate solvent.

Method C.—This method is identical with method B except the final benzene solution which did not yield crystals was evaporated to dryness and the residue recrystallized from a more non-polar solvent.

LAS VEGAS, NEW MEXICO

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, KUNGL. VETERINÄRHÖGSKOLAN]

Radioactive Tetracycline

BY TORSTEN ANDRÉ AND SVEN ULLBERG

RECEIVED JUNE 6, 1956

A method for preparation of tritium-labeled tetracycline of high specific activity is described. The H^3-tetracycline is produced by catalytic hydrogenation of chlorotetracycline with tritium gas in a microhydrogenator with the capacity of 1.5 ml.

The synthesis of radioactively labeled antibiotics has greatly increased the possibilities for investigation of the pharmacology and mode of action of these drugs. Tetracycline has become of foremost importance among the broad spectrum antibiotics and its stability is of considerable advantage in tracer studies.

We have been unable to find in the literature any report of a method for synthesis of radioactive tetracycline.

There are two main alternatives for labeling tetracycline: labeling with C^{14} and H^3. It would be possible to label tetracycline with C^{14} using biosynthetic methods. However, the product would not have a sufficiently high specific activity for many biological investigations unless large amounts of labeled precursor were used and that would make the method prohibitively expensive.

These disadvantages can be avoided by using tritium. The use of tritium offers additional advantages in autoradiography since its particularly short radiation range (energy, 0.017 m.e.v.) provides unique possibilities in obtaining good resolution. This was of particular importance to the authors who desired to extend their previous studies[1] on the distribution of antibiotics in the body using autoradiography.

The authors have modified the method used commercially for large scale production of tetracycline by catalytic hydrogenation of chlorotetracycline.[2,3] The reaction was performed on a micro-scale using a microhydrogenator. The conditions of the reaction have been altered to permit efficient utilization of tritium in the reaction.

Boothe[2] and Conover[3] used palladium as a catalyst. The present authors preferred platinum oxide which is equally efficient as a catalyst but absorbs less hydrogen. It is essential to use a solvent which does not contain hydrogen (protium) which will freely exchange with the tritium. In dioxane all the hydrogen atoms are bound directly to carbon, and dioxane therefore should fulfill this requirement.

Reaction conditions had to be further modified because of the isotope effect. The heavier isotope tritium may react considerably slower than the lighter protium. The difference in reaction rate between tritium and deuterium is much smaller. Therefore deuterium was used to flush the hydrogenation apparatus before introducing the tritium and at the end of the reaction with tritium, deuterium was introduced in small amounts to complete the hydrogenation.

Since the reaction is irreversible the utilization of tritium is enhanced by prolonging the reaction time and by using a reduction mixture as rich in tritium as possible.

Before the product is used as a tracer the exchangeable tritium must be removed.

Procedure.—A hydrogenation apparatus with the capacity of 1.5 ml. was used. One hundred mg. of chlorotetracycline base was dissolved in 1.2 ml. of dioxane in the hydrogenation bottle. Three mg. of platinum oxide was used as a catalyst. An equivalent amount of triethylamine was added to combine with the hydrochloric acid

(1) S. Ullberg, *Acta Radiol.*, Suppl. 118 (1954).

(2) J. H. Boothe, J. Morton, II, J. P. Petisi, R. G. Wilkinson and J. H. Williams, THIS JOURNAL, **75**, 4621 (1953).

(3) L. H. Conover, W. T. Moreland, A. R. English, C. R. Stephens and F. J. Pilgrim, *ibid.*, **75**, 4622 (1953).

formed during the reaction. The reaction mixture was frozen in carbon dioxide and the hydrogenation apparatus was evacuated and flushed several times with deuterium gas before it was finally evacuated. The reaction took place at room temperature. The tritium gas (2 Curie, T_2/H_2 ratio = 78%, volume 1.09 ml., Harwell) was allowed to react first for 1 hour, then small volumes of deuterium gas were introduced at intervals into the reaction bottle so that the hydrogenation was completed.

The catalyst was filtered off and washed three times with 0.1 ml. of dioxane. The filtrate was diluted with 15 ml. of distilled water. During vigorous stirring the *p*H of 7.2 was adjusted to 4.6 with 0.2 ml. of 0.1 *N* sulfuric acid. The stirring was continued for four hours. The solution was kept at +4° overnight and filtered the following day. The filtrate was washed twice with 0.5 ml. of distilled water.

To remove freely exchangeable tritium the product was dissolved in 15 ml. of distilled water and acidified to *p*H 2 with sulfuric acid. After stirring for ten minutes the base was recrystallized by adjusting the *p*H to 4.6 with dilute sodium hydroxide.

The yield was 28 mg. of tetracycline base. The product was analyzed by ultraviolet spectrophotometry.[4] The remaining chlorotetracycline was determined fluorimetrically.[5]

Result.—Tetracycline 98.14%, chlorotetracycline 1.86%. Radioactivity measurement of the tetracycline as an infinitely thin layer was performed in a Tracerlab SC 16 windowless gas flow counter. Comparison with a tritium standard measured under identical conditions resulted in a specific activity of 0.25 mC./mg.

We are indebted to Eng. H. Thelin and Civ. Eng. L. Nathorst Westfelt, AB Astra, Södertälje, for valuable help.

The investigation has been financially supported by a grant from the Knut and Alice Wallenberg Foundation.

(4) "United States Pharmacopoeia," Vol. XV, p. 725.
(5) B. Örtenblad, personal com.

STOCKHOLM, SWEDEN

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF HARVARD UNIVERSITY AND THE CHANDLER LABORATORY OF COLUMBIA UNIVERSITY]

The Stereospecific Synthesis of *dl*-Alloyohimbane and *dl*-3-Epialloyohimbane[1]

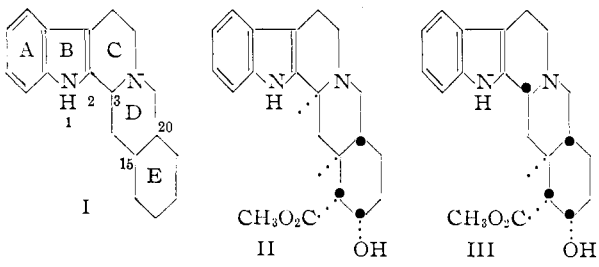
BY GILBERT STORK AND RICHARD K. HILL

RECEIVED JULY 30, 1956

A general method for the stereospecific synthesis of the alloyohimbane skeleton is described. This has led to the first synthesis of the pentacyclic ring system present in reserpine. The stereochemistry of the two alloyohimbanes is established.

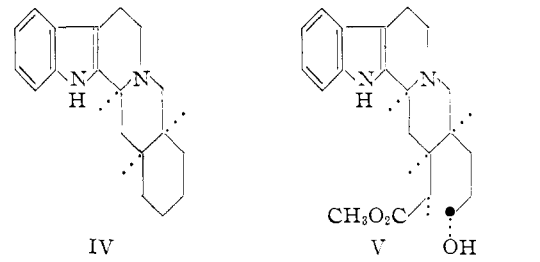
This paper reports the establishment of the stereochemistry of alloyohimbane (IV) and 3-epialloyohimbane (VI) and the development of a stereospecific synthetic route to the pentacyclic nucleus present in reserpine and related alkaloids.

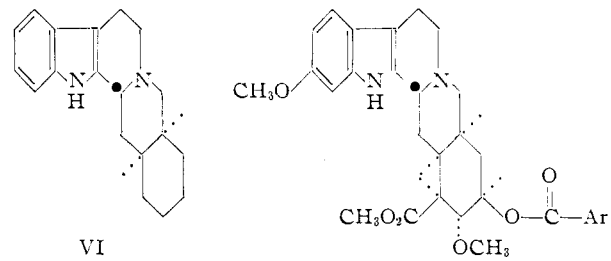
At the time this work was reported[1] only three of the four possible stereochemical arrangements of the pentacyclic system (I) had been encountered in nature. In two of these the junction between rings D and E is *trans* and the two possible configurations at C_3 can be illustrated by yohimbine (II) and ψ-yohimbine (III).[2]



The other two possible arrangements of the skeleton shown in I have a *cis* fused D/E system and again two possible configurations at C_3. The alloyohimbane system (IV) is found, for instance, in alloyohimbine and α-yohimbine (V).

Remarkably, the system of the 3-epimer of IV, which we have termed 3-epialloyohimbane (VI), was encountered in nature only after we had reported its synthesis[1]: its presence was demonstrated by Bader, *et al.*,[3] in "alkaloid 3078" isolated from *Rauwolfia serpentina* and eventually identified as 3-epi-α-yohimbine. Later, deserpidine and reserpine (VII) were identified as members of the new series by MacPhillamy, Huebner, Schlittler, St. André and Ulshafer.[4]





VII, Ar = 3,4,5-trimethoxyphenyl

We now turn to the problem of the stereospecific synthesis of IV and VI and note that the only

(1) G. Stork and R. K. Hill, THIS JOURNAL, **76**, 949 (1954).
(2) For a recent review see J. E. Saxton, *Quart. Rev.*, **10**, 108 (1956).
(3) F. E. Bader, D. F. Dickel, C. F. Huebner, R. A. Lucas and E. Schlittler, THIS JOURNAL, **77**, 3547 (1955).
(4) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André and P. R. Ulshafer, *ibid.*, **77**, 4335 (1955).