The existence of substances which are isotelic with biotin as far as yeast growth is concerned cannot be doubted. For instance, biotin methyl ester, desthiobiotin,[15] and α-biotin of Kögl and co-workers[16] are known to possess biotin activity. These, however, all combine with avidin and hence are not avidin-uncombinable factors. According to results obtained in this Laboratory,[17] repeated tests of the diaminocarboxylic acid derived from biotin, which does not combine with avidin, have shown it to be inactive in the original yeast assay for biotin.[5] This is in contrast to data obtained by others.[4,18] Again, the discrepancy may be due to variations in testing conditions used.

(15) D. B. Melville, K. Dittmer, G. B. Brown and V. du Vigneaud, *Science*, **98**, 497 (1943).

(16) F. Kögl, J. H. Verbeek, H. Erxleben and W. A. J. Borg, *Z. physiol. Chem.*, **279**, 121 (1943).

(17) E. E. Snell, unpublished observations.

(18) V. du Vigneaud, K. Dittmer, K. Hofmann and D. Melville, *Proc. Soc. Expll. Biol. Med.*, **50**, 374 (1942).

Acknowledgment.—We acknowledge with thanks the help of Dr. Roy C. Thompson, who furnished the normal and abnormal rat urine samples.

Summary

When testing conditions such as have been shown to exclude the effects of unrelated yeast nutrilites are used, avidin-uncombinable forms of biotin appear not to be generally distributed in nature, but to be found in urine samples only. The fact that the material which is physiologically active in the yeast test is partially avidin-uncombinable in urine may be due (1) to the presence in urine of products related to biotin which are effective for yeast but do not combine with avidin or (2) to the presence of substances in urine which interfere with the avidin-biotin combination.

AUSTIN, TEXAS RECEIVED MARCH 18, 1944

[CONTRIBUTION FROM THE WM. H. CHANDLER CHEMISTRY LABORATORY, LEHIGH UNIVERSITY]

Experiments in the Synthesis of 3,4-Benzo-9-thiafluorene, a Sulfur Analog of 3,4-Benzophenanthrene

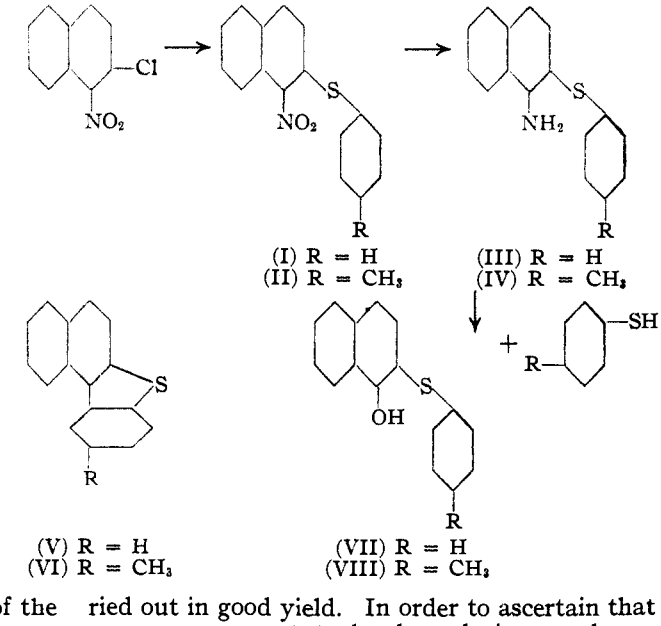
BY C. R. NEUMOYER AND E. D. AMSTUTZ[1]

It has been recognized since the time of Victor Meyer's classical experiments on thiophene that a sulfur atom substituted for a vinylene group of an aromatic carbocycle may cause little change in the physical properties of the compounds. Biological effects of corresponding members in the series often are similar also.[2] This present paper reports on a series of experiments designed to produce the 3,4-benzo-9-thiafluorene nucleus and one of its methyl derivatives.

The ring system is angular and is to be related to 3,4-benzophenanthrene. The isomer 2,3-benzo-9-thiafluorene is linear and is one of the sulfur isologs of the angular hydrocarbon 1,2-benzanthracene. While the 2,3-benzo-9-thiafluorene series is well known, a search of the literature reveals the fact that no member of the 3,4-benzo-9-thiafluorene series has yet been prepared. Our interest in this series of compounds, therefore, concerns not only the possible carcinogenic activity of the substances but also the chemistry of their formation and reactions.

This method of synthesis, as outlined by the flow sheet, seemed to us to be especially desirable since it eliminates the possibility of the formation of isomers.

FLOW SHEET



The condensation[3] of 1-nitro-2-chloronaphthalene with thiophenol or with *p*-thiocresol was carried out in good yield. In order to ascertain that no rearrangement took place during condensation,[4] we prepared an authentic sample of *p*-tolyl-4-nitro-1-naphthyl sulfide and, by the method of mixed melting points, found that this compound and the compound prepared from 1-nitro-2-

(1) Original manuscript received July 21, 1943.

(2) One of the recent correlations of this kind was made by Sandin and Fieser, THIS JOURNAL, **62**, 3098 (1940). It was found (Dunlap and Warren, *Cancer Research*, **1**, 953 (1941)) that 4,9-dimethyl-5,6-benzothiophenanthrene exhibited carcinogenic properties similar to one of the corresponding carbocyclic substances, 9,10-dimethyl-1,2-benzanthracene.

(3) Cullinane, Rees and Plummer, *J. Chem. Soc.*, 151 (1939).

(4) *Cf.* Hodgson and Leigh, *ibid.*, 1031 (1938); also 1094 (1939).

chloronaphthalene and *p*-thiocresol were not identical.

The reduction of phenyl 1-nitro-2-naphthyl sulfide and the corresponding *p*-tolyl compound was attempted by various methods but that using stannous chloride–hydrogen chloride in acetic acid solution[5] is to be preferred not only because of its good yield but also because of the ease of purification of the resulting amine. During the course of the reduction some splitting of the sulfide must have taken place since in each case there was a pronounced odor of the corresponding thiophenol.

The ring-closure reaction[3] (a modification of Pschorr's process) was carried out by diazotization of the amine with subsequent refluxing in 50% sulfuric acid solution. One of the products isolated in minute amount was the disulfide of the corresponding thiophenol which must have been produced during the diazotization or the refluxing since the amines used in this reaction had been purified through their hydrochlorides and could therefore be assumed to be free of the thiophenol. The disulfide collected in the condenser and in the case of the phenyl compound the diphenyl disulfide was identified by a mixed melting point determination with an authentic sample made by oxidation of an alcoholic ammonia solution of thiophenol. Most of the product was obtained as a black tar which solidified on cooling and was very soluble in ether, benzene and acetic acid. This tarry mixture was separated by extraction with ligroin followed by chromatography of the ligroin solution over Brockmann alumina. The resulting chromatogram was developed with a 50% (by vol.) solution of ligroin in benzene after which each band was extracted with ether. The two uppermost bands contained the dark colored impurities which were not further investigated. The third band contained the corresponding phenyl and *p*-tolyl 1-hydroxy-2-naphthyl sulfide, the former being obtained in 1% yield and the latter in 6.5% yield. In both cases the lowest band contained a pale yellow compound which was insoluble in Claisen alkali. The compound from phenyl 1-amino-2-naphthyl sulfide after twice repeated recrystallization from about 75% aqueous acetic acid melted at 174.2–175.7°[6] and was obtained in 0.4% yield. Since repeated recrystallization resulted in considerable loss we attempted to further purify the compound by rechromatography over alumina. This operation yielded only an uncrystallizable oil. Vacuum sublimation lowered the melting point and did not improve the melting range (170.1–172.2°). The substance from *p*-tolyl 1-amino-2-naphthyl sulfide melted at 221.4–225.4° and was obtained in only minute amount. Neither compound was found to exhibit pro-oxidant properties on lard.[7] The analysis of the phenyl compound for sulfur did not check the calculated value. The phenyl compound was oxidized with hydrogen peroxide in glacial acetic acid solution on the water-bath and the analysis of this product likewise did not check the sulfur value calculated for the sulfone, both analyses being low by a fairly constant amount.

The neutral substance isolated from the lowest bands of the chromatograms cannot be the sulfides formed by replacement of the diazonium groups by H since those compounds are known to be distinctly different. Phenyl-2-naphthyl sulfide melts at 51.8°[8a] and *p*-tolyl-2-naphthyl sulfide melts at 70.5°.[8b] Moreover, both are colorless. It is possible that our products represent mixtures of the desired compounds with impurities low in sulfur but the matter cannot be settled definitely until such time as it will be possible to return to projected work in the series.

The ring closure reaction was also found to give very inconsistent results; a total of 39.8 g. of phenyl 1-amino-2-naphthyl sulfide yielded 235.5 mg. of the supposed 3,4-benzo-9-thiafluorene, of which 232.5 mg. was obtained in one reaction starting with 12.9 g. of amino compound.

Attempts to carry out the ring closure reaction by several other modifications[9,10] were unsuccessful.

We concluded that the poor and inconsistent yields from the various modifications of the Pschorr synthesis might possibly be due to failure of the diazotization step. Since good yields are usually obtained in a Sandmeyer reaction, we decided to prepare phenyl 1-chloro- and phenyl 1-bromo-2-naphthyl sulfide from the corresponding amine by this reaction.[11] In our opinion we could not expect a better yield in the ring closure reaction than that obtained in the Sandmeyer. The chloro compound was obtained in 12.5% yield and the bromo compound in 8.7% yield. In each case an appreciable amount of an amine was recovered by treatment of the ether solution of the product with dry hydrogen chloride.

Having obtained the two halogeno compounds, we attempted a ring closure reaction on each with anhydrous aluminum chloride in carbon disulfide solution. The chloro compound was recovered unchanged and the bromo compound yielded a tar which on chromatographic adsorption produced an oil that could not be made to crystallize.

A third method which seemed to be of possible value was a modified Ullmann reaction on *o*-bromo-*p*-tolyl 1-chloro-2-naphthyl sulfide (from 1-nitro-2-chloronaphthalene and 2-bromo-4-methylthiophenol) since this also eliminates the possibility of the formation of isomers.

(5) Cullinane, Davies and Davies, *J. Chem. Soc.*, 1435 (1936).

(6) It is interesting to note that this melting point falls on a practically straight line obtained by plotting melting point *vs.* molecular weight for the following compounds: thiophene (mol. wt. 84; m. p. −37°), benzothiophene (mol. wt. 134; m. p. 31°), and 4,5-benzothionaphthene (mol. wt. 184; m. p. 108–109°).

(7) Lisle, *J. Soc., Chem. Ind.*, **61**, 148 (1942).

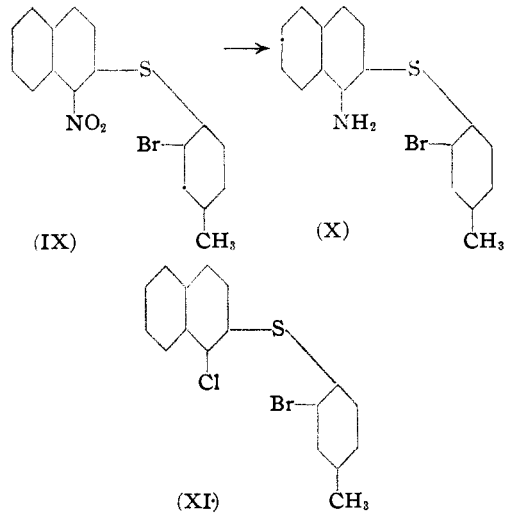
(8a) Bourgeois, *Ber.*, **28**, 2312 (1895).

(8b) Bourgeois, *ibid.*, **24**, 2264 (1891).

(9) Ruggli and Staub, *Helv. Chim. Acta*, **20**, 37 (1937).

(10) Huntress, Pfister and Pfister, THIS JOURNAL, **64**, 2849 (1942).

(11) Hodgson and Walker, *J. Chem. Soc.*, 1620 (1933).



o-Bromo-*p*-tolyl 1-nitro- and *o*-bromo-*p*-tolyl 1-amino-2-naphthyl sulfide were obtained in good yields by reactions analogous to those for (II) and (IV), respectively. A Sandmeyer reaction on the amino-compound gave no product which could be identified as the dihalogen compound (XI).

The poor yields of supposed ring closed compounds from the modified Pschorr synthesis are in accord with the anomalous reactions of the amino compound in the Sandmeyer reaction. The amino-sulfides are relatively unstable, oxidizing easily in air and undergoing gradual change on storage under usual laboratory conditions. The poor yields obtained in the Sandmeyer reaction and the isolation of a considerable quantity of an amine (m. p. 213.9–216.8°) which analyzed correctly for phenyl 1-amino-2-naphthyl sulfoxide, is also added evidence for the anomalous behavior of the corresponding amino-sulfide on diazotization. In the ring-closure reactions of both phenyl and *p*-tolyl compounds the yield of hydroxy sulfides greatly exceeded that of the desired substances, indicating hydrolysis of the diazonium compounds. These reactions plus the fact that diphenyl disulfide and di-*p*-tolyl disulfide were isolated indicates that the Pschorr reaction is only one of perhaps many competing reactions.

Experimental

The corrected melting points have been recorded in all cases and were determined in a Thiele melting point tube.

Phenyl 1-Nitro-2-naphthyl Sulfide (I).—1-Nitro-2-aminonaphthalene[12] was prepared in an over-all yield of 51.3%. After recrystallization from alcohol, it melted at 125.1–127.1°. This was converted into 1-nitro-2-chloronaphthalene,[11] 28.2 g. (0.15 mole) of 1-nitro-2-aminonaphthalene yielding 20.2 g. of the compound which melted at 98.7–100.2° (lit.,[13] m. p. 99–100°), after recrystallization from alcohol. Using an adaptation of the method of Cullinane,[3] 21.1 g. (0.102 mole) of 1-nitro-2-chloronaphthalene and 13.7 g. (0.125 mole) of thiophenol were dissolved in 139 ml. of hot alcohol and a concentrated aqueous solution of 7.0 g. (0.130 mole) of potassium hydroxide added, after which the solution was refluxed twelve hours in an atmosphere of nitrogen. After cooling in a refrigerator overnight, the precipitate was filtered off and washed successively with water, dilute aqueous sodium hydroxide and water. The crude product weighed 28.4 g. (99%) and after recrystallization from 222 g. of alcohol containing Darco, the brilliant yellow compound weighed 24.6 g. (86%) and melted at 58.5–59.5° (lit.,[4] m. p. 58–58.5°).

Phenyl 1-nitro-2-naphthyl sulfoxide was prepared in 71% yield from the corresponding sulfide by oxidation with a 30% solution of hydrogen peroxide (10% excess) in glacial acetic acid solution at room temperature. The compound melted at 80.5–82.0° after recrystallizing twice from alcohol.

Anal. Calcd. for $C_{16}H_{11}O_3NS$: S, 10.78. Found: S, 10.54.

Phenyl 1-nitro-2-naphthyl sulfone was also prepared from the corresponding sulfide in quantitative yield by oxidation with an excess of hydrogen peroxide in glacial acetic acid solution at the temperature of the boiling water-bath. After recrystallization from glacial acetic acid, the compound melted at 147.8–149.2°.

Anal. Calcd. for $C_{16}H_{11}O_4NS$: S, 10.23. Found: S, 10.34.

p-Tolyl 1-Nitro-2-naphthyl Sulfide (II).—This compound was obtained in 85% yield in a manner similar to that for (I) except that the reaction mixture was filtered hot immediately after refluxing. After recrystallization from alcohol containing Darco, the brilliant yellow compound melted at 105.2–105.9°.

Anal. Calcd. for $C_{17}H_{13}O_2NS$: N, 4.74. Found: N, 4.85, 5.11, 4.87.

p-Tolyl 1-nitro-4-naphthyl sulfide, prepared as above from 4-chloro-1-nitronaphthalene and *p*-thiocresol, was obtained as a red crystalline compound melting at 108.2–110.0° after recrystallization from alcohol. A mixture of this compound with (II) melted at 79–90°.

Anal. Calcd. for $C_{17}H_{13}O_2NS$: S, 10.86. Found: S, 11.14.

Phenyl 1-Amino-2-naphthyl Sulfide (III).—A mixture of 54 g. of stannous chloride in 146 ml. of glacial acetic acid was treated with dry hydrogen chloride until a clear solution was obtained. This solution was then added to a solution of 20 g. of (I) in 80 ml. of glacial acetic acid at 80°. After stirring at this temperature for one hour, the reaction mixture was poured into water and extracted with ether. The ether solution was washed with water and a saturated sodium bicarbonate solution and then dried over anhydrous magnesium sulfate after which the hydrochloride of (III) was precipitated by treatment with dry hydrogen chloride. After filtration, the precipitate was washed as rapidly as possible with dry ether (the hydrochloride being unstable in the presence of moisture) and then treated with a saturated sodium bicarbonate solution. The free amine was extracted with ether and the ether extract dried over anhydrous magnesium sulfate. Evaporation of the ether left 11.3 g. (85.5%, taking into account 5.2 g. of original material recovered from first ether extract) of brown oil which solidified on cooling and scratching and which melted at 57.8–59.3° after recrystallization from aqueous alcohol containing Darco.

Anal. Calcd. for $C_{16}H_{13}NS$: S, 12.76. Found: S, 12.59.

p-Tolyl 1-Amino-2-naphthyl Sulfide (IV).—This compound, prepared in an analogous manner in 76.3% yield, melted at 54.1–55.1° after recrystallization from aqueous alcohol containing Darco.

Anal. Calcd. for $C_{17}H_{15}NS$: N, 5.27. Found: N, 5.29, 5.15.

Attempted Ring Closure by Diazotization of (III).—A suspension of 12.9 g. of (III) in 390 ml. of a 50% (by vol.) solution of sulfuric acid was boiled several minutes and then cooled rapidly. It was diazotized at −10 to 0° with a solution of 5.1 g. of sodium nitrite in 51 ml. of water, stirred for an additional two hours and then refluxed for

(12) Saunders and Hamilton, THIS JOURNAL, **54**, 636 (1932).
(13) Hodgson and Leigh, *J. Chem. Soc.*, 1352 (1937).

seven hours with a solution of 132 ml. of concd. sulfuric acid in 330 ml. of water (ultimate concentration 50%). The resulting oil was extracted with ether and the ether solution, after washing with water, was dried over a large excess of anhydrous magnesium sulfate. The ether was distilled in the presence of the magnesium sulfate and the resulting black residue was extracted with ligroin in a Soxhlet extractor. This solution was chromatographed over Brockmann alumina and the chromatogram was developed with a 50% (by vol.) solution of ligroin in benzene after which the separate bands were extracted with ether. After development, the following four bands were obtained: (1) a black band, (2) a brick-red band, (3) an orange band and (4) a pale yellow band. The material from the upper two bands was not investigated further. The lowest band yielded 232.5 mg. of a yellow compound insoluble in Claisen alkali which melted at 173.7–175.2° after recrystallization from glacial acetic acid.

Anal. Calcd. for $C_{16}H_{10}S$: S, 13.68. Found: S, 11.59, 11.51,[14] 11.39.[14]

The compound from band 4 (above) was oxidized with excess hydrogen peroxide in glacial acetic acid solution on a boiling water-bath for six hours and the resulting yellow compound melted at 258.4–259.7°.

Anal. Calcd. for $C_{16}H_{10}O_2S$: S, 12.04. Found: S, 10.85.

Phenyl 1-Hydroxy-2-naphthyl Sulfide (VII).—The material from the ether extraction of the third chromatographic adsorption band was soluble in Claisen alkali and was obtained in 1% yield from (III). After recrystallization from glacial acetic acid, (VII) was obtained as yellow crystals melting at 161.5–163.0°.

Anal. Calcd. for $C_{16}H_{12}OS$: S, 12.71. Found: S, 12.22, 12.19.

Attempted Ring Closure by Diazotization of (IV).—Approximately 1 mg. of a pale yellow compound melting at 221.4–225.4° was obtained in an analogous manner from 2.5 g. of (IV). This compound, insoluble in Claisen alkali, was not available in sufficient quantity for further investigation.

***p*-Tolyl 1-Hydroxy-2-naphthyl Sulfide (VIII).**—This compound was obtained in a manner similar to that for the isolation of (VII) and, after recrystallization from aqueous acetic acid, the brilliant yellow crystals melted at 131.3–132.1°; yield 6.5%.

Anal. Calcd. for $C_{17}H_{14}OS$: S, 12.04. Found: S, 11.96.

Phenyl 1-Chloro-2-naphthyl Sulfide.—A solution of 10.7 g. (0.0426 mole) of (III) in 130 ml. of glacial acetic acid was added to a solution of 3.3 g. of sodium nitrite in 24 ml. of concd. sulfuric acid at a temperature of 5–15° after which the mixture was stirred for an additional two hours. The diazonium mixture was then added to a solution of 8.5 g. of cuprous chloride[15] in 85 ml. of concd. hydrochloric acid over a period of ten to fifteen minutes. After standing overnight, the solid material was filtered off and extracted with ether after which the ether solution was washed with water and dried over magnesium sulfate. The amine present (2 g.) was precipitated as its hydrochloride by treatment with dry hydrogen chloride. After washing and drying the residual ether solution, it was concentrated to a small volume and 0.8 g. of crystals filtered off and recrystallized twice from aqueous acetic acid. The salmon colored compound melted at 182.2–183.9°.

Anal. Calcd. for $C_{16}H_{11}ClS$: S, 11.84. Found: S, 11.79.

Phenyl 1-bromo-2-naphthyl sulfide (1.4 g., 8.7%) was obtained in an analogous manner (from 12.8 g. of amino sulfide) as a salmon colored compound melting at 208.8–210.7° after recrystallization from glacial acetic acid.

Anal. Calcd. for $C_{16}H_{11}BrS$: S, 10.17. Found: S, 9.95.

The amine isolated above as the hydrochloride (6.3 g.) was found to be practically insoluble in ligroin and alcohol and very soluble in benzene. In the crude state it melted at 179–209° (uncor.) and after purification by dissolution in ether and partial evaporation of the resulting solution it melted at 213.9–216.8°.

Anal. Calcd. for $C_{16}H_{13}ONS$: S, 11.99. Found: S, 12.28.

***o*-Bromo-*p*-thiocresol.**—This compound was prepared in 67% yield from the hydrochloride of *o*-bromo-*p*-toluidine[16] by conversion to the thioxanthate ester[17] and subsequent alkaline saponification in a nitrogen atmosphere. The mercaptan was identified by oxidizing it to the disulfide in alcoholic ammonia solution. After recrystallization from alcohol, the colorless disulfide melted at 99.5–101.0°.[18]

Anal. Calcd. for $C_{14}H_{12}Br_2S_2$: S, 15.86. Found: S, 15.42.

***o*-Bromo-*p*-tolyl 1-Nitro-2-naphthyl Sulfide (IX).**—This compound prepared in 77% yield by the same method as that used for (I), melted at 122.1–123.0° after recrystallization from alcohol.

Anal. Calcd. for $C_{17}H_{12}O_2NBrS$: S, 8.57. Found: S, 8.74.

***o*-Bromo-*p*-tolyl 1-Amino-2-naphthyl Sulfide (X).**—Using the same method as that for (III), this compound was prepared in 78% yield and melted at 83.5–84.5° after recrystallization twice from alcohol.

Anal. Calcd. for $C_{17}H_{14}NBrS$: S, 9.31. Found: S, 9.23.

***o*-Bromo-*p*-tolyl 1-acetamino-2-naphthyl sulfide** was prepared by acetylation of the corresponding amine with acetic anhydride in glacial acetic acid solution and melted at 199.2–200.4° after recrystallization from alcohol.

Anal. Calcd. for $C_{19}H_{16}ONBrS$: S, 8.30. Found: S, 8.32.

Summary

The synthesis of 3,4-benzo-9-thiafluorene and its 6-methyl derivative has been attempted using a modified Pschorr ring-closure on the appropriate amino sulfides. The process results in a mixture of products one of which is possibly the desired thiophene derivative. Another product is the naphthol corresponding to the naphthylamine used.

The synthesis of the parent compound using a Friedel–Crafts reaction on the corresponding bromo sulfide has also been attempted.

The synthesis of several new compounds has been described.

BETHLEHEM, PA. RECEIVED JUNE 20, 1944

(14) Analysis by Dr. Carl Tiedcke.

(15) "Organic Syntheses," Coll. Vol. I, p. 156.

(16) *Ibid.*, p. 106.

(17) Leuckart, *J. prakt. Chem.*, [2] **41**, 179 (1890); *cf.* also Mauthner, *Ber.*, **39**, 3593 (1906).

(18) Zincke and Frohneberg, *Ber.*, **43**, 837 (1910), report that this compound melts at 88° and that its isomer (the disulfide of 3-bromo-4-methyl-thiophenol) melts at 100°. Since there should be no doubt about the structure of the compound prepared by our method, we believe that these authors' compounds (VIII) and (XXI) should be interchanged.