sauf pour celui du composé **11b** dans lequel, malgré un temps de réaction prolongé et une quantité double d'acide picrique il n'a pu dépasser 25%. – Schéma 2.

Par ailleurs, les cétones **5a** et **5b** (0,015 *M*) ont été condensées selon la technique de Friedlaender modifiée par KEMPTER et al.[9,3] soit avec le chlorhydrate d'ortho-aminobenzaldéhyde[10] soit avec le chlorhydrate d'ortho-aminoacétophénone (0,01 *M*) à 140° pendant 1/2 h. La décomposition du mélange réactionnel par l'ammoniaque diluée et la purification du résidu par chromatographie sur gel de silice (élution au benzène) permet d'accéder aux quinoléines **12a** et **12b** (rdt. 60%) et aux quinoléines **13a** et **13b** (rdt. 75%) – Schéma 3.

Les constantes physiques des hétérocycles azotés obtenus dans ce travail sont collationnées dans les Tableaux I et II. Les différentes substances décrites dans ces Tableaux font d'ores et déjà l'objet d'une étude quant à leur éventuelle activité sarcomogène chez la souris par voie sous cutanée[11]; les résultats de ces expérimentations biologiques seront rapportés par ailleurs.

Summary. Some fluoro derivatives of [1]benzothiopyrano[4,3-*b*]indoles and 6H[1]benzothiopyrano[4,3-*b*] quinolines have shown carciuogenic activities in regard to the fluorine position; we thought it interesting to prepare some trifluoromethyl analogs of there compounds to study the biological activities and to compare with other compounds of the same family. In the present work, we report the synthesis of 7-trifluoromethyl and 8-trifluoromethyl [1]thiochroman-4-one and some [1]benzothiopyrano[4,3-*b*]indoles and quinolines substitued in 3 or 4 position which have been obtained from these two ketones.

P. JACQUIGNON, A. FRAVOLINI, A. FERON et A. CROISY

Institut de Chimie des Substances Naturelles du CNRS, 91190 Gif sur Yvette (France); Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi, Perugia (Italie), et Laboratoire de Chimie Macromoléculaire et de Catalyse Organique, Domaine Universitaire du Sart-Tillman, 1B–4000-Liège (Belgique), 19 novembre 1973

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A Hitherto Undescribed Pigment Found in Human Urinary Uric Acid Stones

An accidental observation in urinary stone analysis led to systematic studies of a peculiar pigment. When, in June 1971, I analyzed in a routine way a powdered part of a pea-sized, brickred stone with boiling hydrochloric acid, the stone material remained undissolved, but the fluid was stained pink. Obviously I was dealing with a uric acid stone, because it dissolved completely in boiling alkali. But now the color of the alkaline fluid was amber-yellow. Renewed acidification produced again the former pink coloration and, at the same time, precipitation of large, beautifully pink stained crystals of uric acid (KLEEBERG[1]). I then believed that this dye was urorosein, for two reasons: 1. its excellent solubility in amyl alcohol and 2. the above described color-change dependent on the pH of the medium (KLEEBERG, WARSKI and SHALITIN[2]). Later studies proved this assumption to be wrong. The new analytical approach will be reported here.

Material and methods. The urinary human stones were obtained from various sources. Parts of 18 pure uric acid calculi and of 150 calcium oxalate phosphate stones (whose chemical composition had been determined by routine qualitative analysis) were powdered and boiled with 2–3 ml of an 8% sodium hydroxide solution for at least 1 min, cooled to room temperature and acidified with 3 ml of a 10% hydrochloric acid. Those specimens which produced a distinct yellow color in the alkaline fluid and, which after acidification caused a pink stain, were taken as suitable for further examination. Of the 168 calculi, only 4 uric acid stones showed the desired reaction. Two small concretions were discarded. The remaining two (2.5 g and 2.3 g respectively) were used as the material for the following procedure, which follows essentially a method by KUENZLE[3].

The 2 calculi weighing together 4800 mg were powdered and twice extracted with boiling methanol for 10 min. After cooling the resulting deep yellow fluid was rapidly filtered twice under reduced pressure, and then the combined filtrates evaporated to dryness in a vacuum rotation evaporator at 38°–40 °C. The residue was dissolved in a mixture of 20 ml ethanol-methanol (1:1) at room temperature, filtered and again dried in a vacuum rotation evaporator.

The powdered stone residue from the methanol extraction was re-extracted with absolute ethanol in an identical procedure. The 2 dried orange-yellow residues from the methanol and ethanol solutions were each dissolved in 2 ml of warm absolute ethanol and subjected to thin layer chromatography (TLC) on silica-gel glass plates (E. Merck, 0.25 mm; No. F 254). The eluent was a mixture of chloroform-acetone-ethanol (2:1:1). According to the method of KUENZLE[3] the plates were run for 2 h, dried and re-run for further $1^1/_2$ h.

A very light violet line 1 mm broad appeared at the top of both plates as well as a very faint pink broader zone without definite borders; both bands were discarded. The main chromatographic fraction was a 3 cm broad orange-yellow band. It was removed from the 2 plates and separately extracted with about 30 ml methanol-ethanol (1:1) for several hours at room temperature, using a magnetic stirrer. The fluids were twice filtered under reduced pressure and finally concentrated to about 5 ml in a vacuum rotation evaporator. These deep yellow fluids were used for chemical spot tests and physical examinations. The chemical spot tests were made on filter paper with concentrated mineral acids and glacial acetic acid. Tests were also performed for bilirubin with Fouchet's reagent and with iodine solution; for urobilinogen with Ehrlich's aldehyde reagent and for urobilin with Schlesinger's zinc-azetate reagent.

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The 3 physical examinations, namely maximum absorption spectroscopy (MAS), IR- and mass spectroscopy (MS) were made with the methanol extract only. The ethanol extract was examined for maximum absorption spectroscopy.

Results. Chemical: The deep yellow color of the solution spots was changed to pink by the applied acids. Concentrated nitric acid produced a pink periphery but the centre was stained distinctly blue. Tests for bilirubin, urobilin and for urobilinogen were negative. The pigment was soluble in water, methanol, ethanol and especially in amyl alcohol. There were no differences in the outcome of the described tests between the methanol and the ethanol extracts.

Physical: The MAS of the alkaline fluid was 432 nm and after acidification 476 nm. The IR-spectrum showed CO- and NH-groups; the mass spectrum showed a peak at 229.

Discussion. The analytical procedures proved the presence of a peculiar pigment in only 4 pure uric acid stones and not in the 14 other pure tri-oxy-purine calculi or in the 150 oxalate-phosphate concretions. The 4 patients who had been hospitalized for stone operation had not been treated with color-producing drugs or fed with large amounts of specific food which might have imparted the stain to the concretions. One must, therefore, assume that this peculiar pigment originated from metabolic processes in the human body and was excreted by the kidneys.

Our first assumption[2] that this urinary natural dye in the calculi might be urorosein was based on 3 facts: 1. the easy solubility in amyl alcohol, 2. its pink color in an acid medium and its amber yellow in an alkaline, 3. its property to stain crystals of uric acid pink. Urorosein is a polymer of an α-β-di-indolyl methene (VON DOBENECK[4]). Its MAS of 535 nm, as well as its molecular weight, are against the idea that our pigment is a derivative of indole.

The mass spectrum of 229 excludes all tetrapyrroles (bilirubin, urobilin) as the staining agent. On the other hand, a molecular weight of 229 and the presence of CO and of NH-groups shown by the IR-spectrum might be consistent with the assumption that our pigment could be a di-pyrrole; more specifically of the Neo-type (an oxo-di-pyrrole methene with at least 1 vinyl group (VON DOBENECK[5]). Compounds of such chemical configuration show spectroscopically a shift to higher absorption maxima in an acid milieu than in an alkaline, as has been shown by NICHOL and MORELL[6] for certain bilirubin derivatives. Such a shift is also characteristic for the pigment described here.

There are other di-pyrroles in normal and pathological urines. WITH[7] mentiones urochrome-B, the fuscins and the leukans. Meso-bilifuscin, a di-pyrrole, a brown pigment has been described by MORAVEC[8] to be soluble in glacial acetic acid. However, these di-pyrroles, as well as the pent-dyo-pent and pro-pent-dyo-pent compounds, present quite other chemical and physical properties than does our stone pigment (VON DOBENECK[9]).

Zusammenfassung. Es wird das Vorkommen eines bisher unbekannten Pigmentes in Harnsäuresteinen beschrieben. Chemisch handelt es sich wahrscheinlich um eine Oxo-di-pyrrol-methen-Verbindung.

J. KLEEBERG[10]

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3,4-Dihydroxyphenylacetic acid (DOPAC): a Possible Endogenous Inhibitor of Indoleamine-N-Methylation in the Rat Brain

In recent years, a number of important observations concerning the occurrence and properties of an indoleamine- and catecholamine-N-methylating enzyme in mammalian brain have been reported[1–4]. It seems to be generally accepted that 5-methyltetrahydrofolic acid serves as the methyl donor for this enzymatic reaction. Several N-methylated monoamines have been implicated in the aetiology of psychotic disorders[1,5,6]. Assuming that this hypothesis is valid and that neuroleptic drugs exert their beneficial effect on psychotic disorders by interfering with these substances, there are at least 4 possible ways in which they may act: a) they may inhibit the enzyme directly, b) they may block the receptors on which N-methylated amines possibly act, c) they may stimulate the production or release of endogenous substances which inhibit the enzyme, and d) they may influence the degradation of N-methylated amines.

The aim of the present investigation was to study the effects on indoleamine-N-methylation of 2 well-established neuroleptics and of the 2 endogenous amine metabolites whose concentrations in the brain are most spectacularly increased by the former[7,8].

The enzyme was prepared according to the procedure described by HSU and MANDELL[2], and LADURON[1], the whole brains of 50 male Sprague-Dawley albino rats weighing 200–250 g being used for each batch. The brains

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