compared as a function of substrate concentration (Fig. 5). The activity of bound enzyme was determined by mixing with the substrate solution for 30 sec, using a mechanical mixer, and measuring the absorbance of the supernate after standing for 10 sec. The maximum velocity of the immobilized enzyme was slightly lower than that of the free enzyme, but the K_m values were similar. These properties did not vary on adsorption for 1 week. Hence, the surfaces of silicone-coated glass may be useful as a support for the enzymes to be immobilized, but the surfaces of noncoated glass are not appropriate for this purpose (3).

With the noncoated glass in the column, the top portion of the glass turned milky white after albumin solution was applied whereas no color change was observed when coated glass was used. Albumin might have precipitated on the noncoated glass but was well adsorbed onto the coated glass. The difference in the adsorption forces between amine–silanol ionic bonding and hydrophobic interaction might have caused this difference.

Adsorption on Synthetic Polymers—Synthetic polymers such as polyethylene and polyethylmethacrylate are useful for pharmaceutical containers. However, polyethylene was shown to adsorb 155 mg of human serum albumin/100 m^2 of polyethylene surface (16). Hemoglobin also is adsorbed onto polyethylene (17). Polyethylmethacrylate and polyhydroxyethylmethacrylate adsorb plasma proteins such as hemoglobin, albumin, fibrinogen, and γ-globulin (18). Polystyrene adsorbs human serum albumin, ribonuclease, and muscle proteins (19, 20). The reported amounts of proteins adsorbed onto synthetic polymers are comparable to those adsorbed onto coated and noncoated glass, although the mechanisms of protein adsorption onto synthetic polymers and glass must be different.

Pharmaceutical preparations composed of proteins such as hormones and vaccines adsorb onto the surfaces of their containers. This adsorption is particularly significant at low protein concentrations since at least 7 μg of protein is adsorbed by the surface of a 20-ml glass container. Thus, it is important to compensate for this adsorption when dispensing low doses of such substances.

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Synthesis of Tetracycline Ring A Analogs

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Abstract □ Studies directed at the synthesis of tetracycline ring A analogs are described. 4-Carbethoxycyclohexane-1,3-dione was converted to the ethyl urethan dispiro[1,3-dioxolane-2,2′-cyclohexane-4′,2″(1,3)-dioxolane]-1′-carbamic acid ethyl ester *via* the dispiro[1,3-dioxolane-2,2′-cyclohexane-4′,2″(1,3)-dioxolane]-1′-carboxylic acid hydrazide. An improved synthesis of another cyclohexenone from methyl vinyl ketone and ethyl nitroacetate is reported. Reaction of *N*-(3-hydroxy-1-oxo-2-cyclohexen-4-yl)benzamide with α-chloroacetyl isocyanate afforded a 4(5*H*)-oxazolone derivative, as did the identical reaction on 5,5-dimethyl-1,3-cyclohexanedione (dimedone). This reaction provides a novel approach to these oxazolones with potential therapeutic importance. Other ring A analogs were synthesized also.

Keyphrases □ Tetracycline—synthesis of ring A analogs □ 4(5*H*)-Oxazolones—tetracycline ring A analogs, synthesis □ Analogs—tetracycline ring A analogs, synthesis

Following a study of the mechanism of the polarographic reduction of tetracycline compounds (1), the need arose for simpler analogs encompassing some or all structural features of ring A of the tetracyclines. A literature search, followed by some preliminary attempts at duplicating one synthetic approach, revealed that a ready access to these compounds had not been published.

Smissman *et al.* (2) attempted, without success, to prepare ring A analogs by cyclizing appropriate alicyclic molecules. Muxfeldt *et al.* (3) prepared several ring A analogs by the condensation of unsaturated oxazolones with active methylene compounds. Compound I was obtained, which could be hydrolyzed and decarboxylated to give II. Alternatively, treatment of I with Meerwein's reagent afforded III (Scheme I).

A different approach was reported (4, 5) for preparing ring A analogs, involving catalytic hydrogenation of substituted resorcinols to yield the corresponding 1,3-cyclohexanedione derivatives. All efforts at duplicating this synthesis were unsuccessful. Several reported approaches

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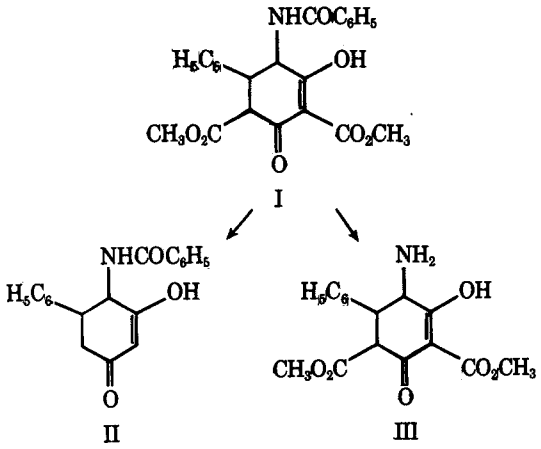


Scheme I

lead to two-ring compounds that include some features of the B as well as the A ring of the tetracyclines (6, 7).

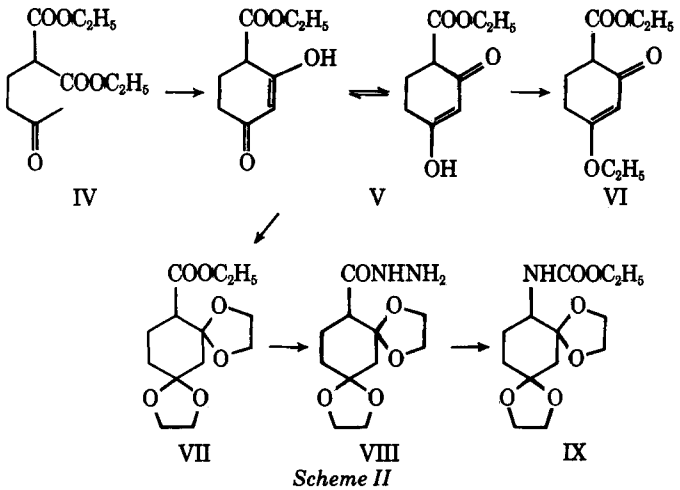
This paper reports the results from studies directed at synthesizing some ring A analogs of the tetracyclines.

RESULTS AND DISCUSSION

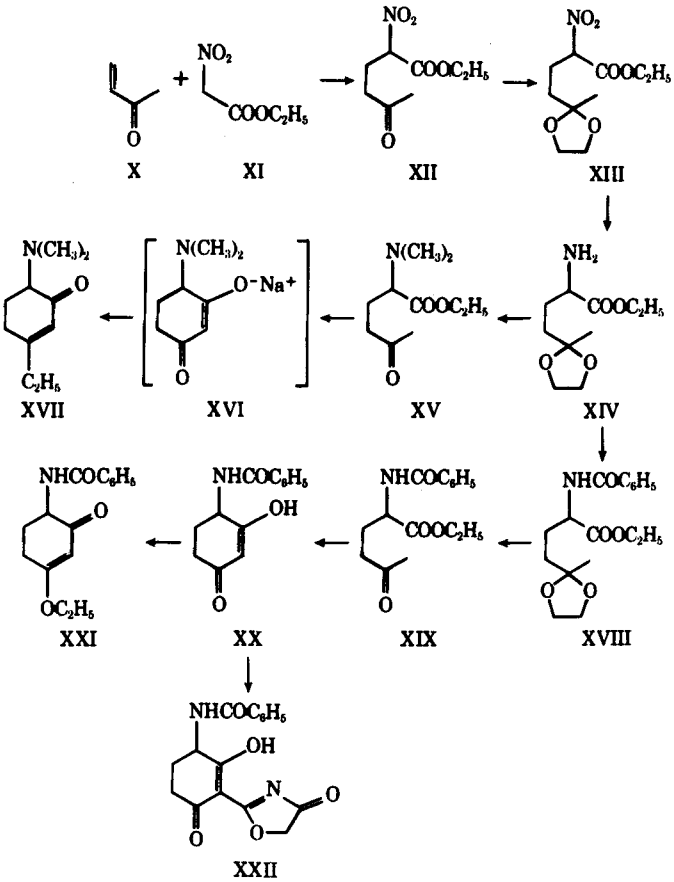
The initial synthetic approach to the ring A analogs started with the previously reported 4-carbethoxycyclohexane-1,3-dione (V) (8). This compound was prepared by cyclization of the keto ester (IV), using sodium hydride in anhydrous benzene to effect the cyclization. Spencer *et al.* (8) employed sodium ethoxide in ethanol as the cyclizing agent and obtained variable yields. The enol ether (VI) often was isolated as the major product, arising presumably from the further reaction of ethoxide ion on the cyclized product. An interesting feature of the NMR spectrum of V was the two overlapping triplet–quartet ethyl systems separated by δ <0.1. This observation is consistent with proton tautomerism as depicted in Scheme II.

Ketalization of V was carried out in the usual manner in ethylene glycol and benzene with a catalytic amount of *p*-toluenesulfonic acid to give VII. Crude VII was used without further purification and was treated with hydrazine hydrate to form the hydrazide derivative (VIII). The hydrazide was converted to the ethyl urethan (IX) en route to its conversion to the amine. Difficulty was encountered with the attempted hydrolysis of the urethan; invariably, the β-dicarbonyl system was destroyed under the conditions necessary to effect hydrolysis. Since an alternative entry to the amino group seemed promising, further attempts at hydrolysis of the urethan were discontinued.

In the alternative route, the amino function was introduced at C-4 by catalytic hydrogenation of the intermediate nitro compound, affording the key intermediate (XIV) (Scheme III). Ethyl nitroacetate (XI) was prepared by the method of Rodionov *et al.* (9). Addition of this active methylene compound to the activated double bond in methyl vinyl ketone (X) was accomplished in good yield using tributyl phosphine as the cat-



Scheme II



Scheme III

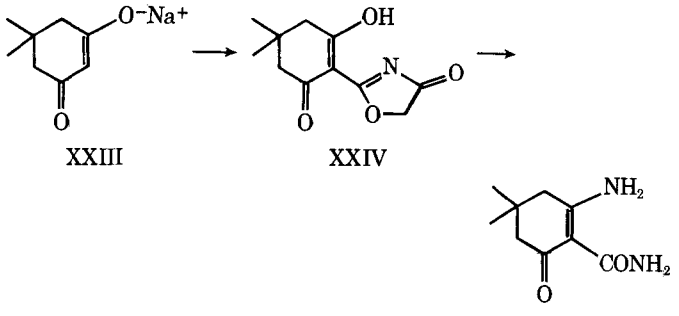
alyst. Protection of the carbonyl group by ketal formation was followed by reduction with 10% palladium-on-charcoal at 1000–1500 psi. The amine (XIV) could be reductively alkylated in excellent yield by treatment with excess formaldehyde and hydrogen at 40 psi using the same catalyst. The ketal protective group was removed in the usual manner by acid hydrolysis, and the compound was characterized as ketone XV.

When cyclization of XV was attempted with sodium hydride in anhydrous benzene, an amorphous precipitate was obtained after 10 hr of refluxing. This precipitate was the sodium salt of 3-hydroxy-4-dimethylamino-2-cyclohexen-1-one (XVI). Attempts to isolate the free acid by adding dilute hydrochloric acid, adjusting the pH to 8–9, and extracting with ether failed. At this point, the aqueous solution was made acidic (pH 2) and evaporated to dryness *in vacuo*. The resultant mass was extracted with absolute ethanol and, after workup, afforded XVII.

The problems encountered in attempting to isolate the free acid of XVI appeared to be associated with its amphoteric nature. Therefore, another approach was tried involving conversion of the basic amino moiety to a neutral species, followed by cyclization and subsequent removal of the protective group. The benzoylamino group was chosen since conditions for its hydrolysis have been well documented (10–12). The amino ketal ester (XIV) was converted to the benzoylamino derivative (XVIII) by treatment with benzoyl chloride. Acid hydrolysis at room temperature afforded XIX. Crude XIX was cyclized to XX with sodium hydride in anhydrous benzene. Compound XX had been prepared previously by another synthetic route (4).

Attempted removal of the benzoyl protective group by the procedure described by Muxfeldt *et al.* (13) gave XXI. The isolation of this *O*-alkylated product was in agreement with experiments conducted by Durckheimer (14), who employed the same reagent to effect etherification at position 1 (or 3) of some tetracyclines. Since all compounds studied by Muxfeldt *et al.* (13) contained a substituent at C-2, further attempts at removing the protective group were postponed until a substituent could be introduced at C-2.

Carboxamidation experiments with potassium cyanate failed to yield any products capable of being isolated. Since acetyl isocyanate is difficult to prepare, it was decided to try the more readily accessible α-chloroacetyl isocyanate (15). Initial experiments with the sodium enolate of 5,5-dimethyl-1,3-cyclohexanedione (dimedone) (XXIII) (Scheme IV) gave



XXIII XXIV XXV

Scheme IV

the unexpected product XXIV. Compound XXIV exhibited UV behavior that was characteristic of enolic β-diketones. In basic solution, a bathochromic shift and a hyperchromic (16) effect were observed, which could be reverted by neutralization with acids. This compound contained the 4(5*H*)-oxazolone system, the spectral properties of which were reported previously (17). This reaction represents a novel approach to the synthesis of 4(5*H*)-oxazolones, which potentially are of therapeutic importance. Interest in this class of compounds has grown recently, particularly in the 2-amino derivatives; they have found use as tranquilizers, antidepressants, memory aids, and appetite suppressants (18). The antibiotic indolmycin contains the 4(5*H*)-oxazolone system (19).

Since the 4(5*H*)-oxazolone ring system in XXIV is strained (carbonyl absorption is at 1770 cm^{-1}), it was thought that it might collapse under rather mild conditions. Hence, treatment of XXIV with saturated ammonia in methanol at room temperature gave what appeared to be XXV. The high-resolution mass spectrum was consistent with this structure. The only perplexing feature preventing a conclusive structural assignment was the four-proton methylene singlet at δ 2.4 in the NMR spectrum. There was no evidence of any resolution in deuteroacetic acid, deuterochloroform, deuteropyridine, or deuterotrifluoroacetic acid. When α-chloroacetyl isocyanate was allowed to react with XX, the 4(5*H*)-oxazolone (XXII) was obtained.

EXPERIMENTAL[1]

Melting points[2] and boiling points are uncorrected. IR spectra[3] were recorded as thin films or as potassium bromide disks. UV spectra[4] were taken as solutions in ethanol. NMR spectra[5] were taken in deuterated solvents from commercial sources with tetramethylsilane as the internal standard. Mass spectra[6] were determined using the direct-probe technique.

Dispiro[1,3-dioxolane-2,2′-cyclohexane-4′,2″(1,3)-dioxolane]-1′-carboxylic Acid Ethyl Ester (VII)—A stirred mixture of V (18.5 g, 0.1 mole), benzene (250 ml), ethylene glycol (100 g, 1.6 moles), and *p*-toluenesulfonic acid (0.5 g) was heated to reflux for 2 days in a round-bottom flask fitted with a Dean–Stark water separator. The benzene layer was separated, and the glycol layer was extracted with ether (3 × 40 ml). The benzene and ether extracts were combined and washed with 10% sodium carbonate followed by water. The organic layer was dried and evaporated to dryness under reduced pressure (20.3 g, 74%). An analytical sample was obtained through TLC [benzene–ethanol (9:1), silica gel]; $n_D^{24.4}$ 1.4826; IR (neat): 2980, 2790, 1730 (ester carbonyl), 1450, 1370, 1180, 1080, 1030, 950, and 825 cm^{-1}; NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 6–7 Hz, OCH_2CH_3), 1.5–3.1 (m, 7H, cyclohexane ring protons), and 4.0–4.4 [m, 10H, OCH_2CH_3 and $2(OCH_2CH_2O)$].

Anal.—Calc. for $C_{13}H_{20}O_6$: C, 57.34; H, 7.40. Found: C, 57.77; H, 7.46.

Dispiro[1,3-dioxolane-2,2′-cyclohexane-4′2″(1,3)-dioxolane]-1′-carboxylic Acid Hydrazide (VIII)—Hydrazine hydrate (40 ml, 0.6 mole) in anhydrous ethanol (20 ml) was stirred magnetically and heated to reflux. The diketal ester (VII) (27.2 g, 0.1 mole) in anhydrous ethanol (20 ml) was added dropwise over 0.5 hr. The solution was refluxed for 30 hr, the ethanol was removed at reduced pressure, and the remaining solution was evaporated to dryness *in vacuo*. The residual oil was dried in a vacuum desiccator for 2 days and then triturated with anhydrous ethanol to give 25 g of the crude product.

Recrystallization from ethanol afforded VIII, mp 115–116°; IR (KBr): 3400, 3300, 3200 (N–H), 1670 (amide carbonyl), 1510 (N–H binding), 1370, 1210, 1150, 1090, 1070, 1060, 1040, 950, 830, 800, and 740 cm^{-1}; NMR ($CDCl_3$): δ 1.6–2.2 (m, 6H, $3CH_2$), 2.52 [t, 1H, J = 6 Hz, $O{=}C(NHNH_2)CH$], 3.68 (broad s, 2H, $O{=}CNHNH_2$), 3.93 [d, 8H, J = 3 Hz, $2(OCH_2CH_2O)$], and 7.64 (broad s, 1H, $O{=}CNHNH_2$); mass spectrum: parent ion m/z 258.1214 (53.8%) (calc. for $C_{11}H_{18}NO_3$: 258.1211), 259 (7.2), 199 (13.7), 172 (26.1), 141 (50.8), 113 (49.1), 99 (98.7), 87 (63.4), 86 (45.8), 69 (26.1), and 55 (100).

Dispiro[1,3-dioxolane-2,2′-cyclohexane-4′,2″(1,3)-dioxolane]-1′-carbamic Acid Ethyl Ester (IX)—The hydrazide (VIII) (2.2 g, 8.5 mmoles), dissolved in 6 *N* HCl (3 ml), 5% acetic acid (5 ml), and ether (25 ml), was chilled to 0–5° and stirred magnetically. A solution of sodium nitrite (0.6 g in 1 ml of water, 8.5 mmoles) then was added at such a rate that the temperature did not rise above 10°. After addition was complete, the ether layer was separated and the aqueous solution was extracted four times with ether. The combined extract was washed twice with a saturated sodium bicarbonate solution and twice with water, dried, and filtered. To the filtrate was added an excess of anhydrous ethanol. The ether was removed by distillation, and the resultant ethanolic solution was refluxed for 4 hr.

The ethanol was evaporated under reduced pressure, and the viscous liquid residue solidified on cooling (1.85 g, 75%). Recrystallization from chloroform afforded IX, mp 124°; IR (KBR): 3340 (N–H stretching), 2980, 2790, 1720 (urethan carbonyl), 1545, 1330, 1250, 1150, 1080, 1060, 1030, 950, and 830 cm^{-1}; NMR ($CDCl_3$): δ 1.26 (t, 3H, J = 7 Hz, OCH_2CH_3), 3.7–4.3 [m, 10H, $2(OCH_2CH_2O)$ and OCH_2CH_3], and 4.8 [broad s, 1H, $NH(CO_2C_2H_5)$]; mass spectrum: m/z 288 (0.6%), 287 (1.5), 201 (63.9), 187 (14.1), 173 (26.4), 159 (27.2), 157 (73.3), 100 (29.2), 99 (54.1), 87 (70.7), 86 (100), 73 (33.5), 57 (31.7), and 55 (27.7).

Anal.—Calc. for $C_{13}H_{21}NO_6$: C, 54.34; H, 7.36; N, 4.81. Found: C, 53.99; H, 7.28; N, 4.92.

2-Nitro-5-oxohexanoic Acid Ethyl Ester (XII)—To a stirred solution of ethyl nitroacetate (XI) (13.3 g, 0.1 mole) and methyl vinyl ketone (X) (7 g, 0.1 mole) in tetrahydrofuran (50 ml) at room temperature was added dropwise a catalytic amount of tributyl phosphine. The heat evolved was sufficient to bring the solution to a gentle reflux. After the solution had returned to room temperature (~2 hr), methyl iodide was added to remove the phosphine. Direct distillation yielded XII (16 g, 78%), bp 134–138°/3.5 mm; $n_D^{23.2}$ 1.4473; IR (neat): 2980, 2960, 2940, 2910, 2870, 1750, 1720, 1560 (nitro stretching), 1370, 1260, 1170, 1020, and 860 cm^{-1}; NMR ($CDCl_3$): δ 1.28 (t, 3H, J = 7 Hz, OCH_2CH_3), 2.14 (s, 3H, $COCH_3$), 4.25 (q, 2H, J = 7 Hz, OCH_2CH_3), and 5.26 [t, 1H, $CH(NO_2)CO_2C_2H_5$].

Anal.—Calc. for $C_8H_{13}NO_5$: C, 47.28; H, 6.44; N, 6.89. Found: C, 47.23; H, 6.40; N, 6.81.

2-Methyl-2-(α-nitrobutanoic acid)-1,3-dioxolane Ethyl Ester (XIII)—A stirred mixture of XII (15 g, 74 mmoles) in benzene (160 ml), ethylene glycol (100 ml), and *p*-toluenesulfonic acid (0.5 g) was heated to reflux under a Dean–Stark trap for 24 hr. The mixture was extracted with ether (3 × 80 ml) and washed once with 10% sodium bicarbonate and once with water. The ether was dried, filtered, and evaporated *in vacuo*. The resulting liquid was slowly and carefully vacuum distilled to yield a pale-yellow liquid (12 g, 68%), bp 112–116°/2 mm; $n_D^{20.0}$ 1.4547; IR (neat): 2980, 2960, 2930, 2880, 1750, 1560 (nitro stretching), 1450, 1375, 1260, 1060, 1040, 950, and 860 cm^{-1}; NMR ($CDCl_3$): δ 1.33 (t, 6H, J = 7 Hz, terminal methyl and OCH_2CH_3), 3.96 (s, 4H, OCH_2CH_2O), 4.3 (q, 2H, OCH_2CH_3), and 5.28 [t, 1H, J = 7 Hz, $CH(NO_2)CO_2C_2H_5$]; mass spectrum ($M^+ - CH_3$): 232.0820 (14.9%) (calc. for $C_{10}H_{17}NO_6 - CH_3$: 232.0820), 99 (14.8), 87 (100), and 86 (24.3).

Anal.—Calc. for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.93; N, 5.67. Found: C, 49.64; H, 7.10; N, 5.78.

2-(α-Aminobutanoic acid)-2-methyl-1,3-dioxolane Ethyl Ester (XIV)—Compound XIII (20.3 g, 85 mmoles) was mixed with 5 g of 10% palladium-on-charcoal in 95% ethanol (150 ml). Hydrogenation was carried out under 1500 psi at 50° for 10 hr. The catalyst was filtered, and the solvent was removed by evaporation. The liquid residue was dissolved in benzene, dried, and finally evaporated to give an oily liquid (17 g, 93%), which solidified on standing after a few days at room temperature. An analytic sample had a melting point of 212–214° dec.; IR (KBr): 3200, 3080, 3030 (N–H stretching), 2980, 2880, 1670, 1450, 1380, 1330, 1250, 1220, 1140, 1065, 1050, 950, and 870 cm^{-1}; NMR ($CDCl_3$): δ 1.0–1.5 (m, 6H, terminal methyl and OCH_2CH_3), 2.25 [s, 2H, $CH(NH_2)CO_2C_2H_5$], 3.90 (s, 4H, OCH_2CH_2O), and 4.17 (q, 2H, J = 7 Hz, OCH_2CH_3).

Anal.—Calc. for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.81; N, 6.44. Found: C, 55.27;

[1] Microanalyses were performed by the Microanalytical Laboratory, Chemistry Department, University of Alberta.
[2] Thomas-Hoover capillary apparatus.
[3] Unicam SP1000 or Perkin-Elmer model 267 grating IR spectrophotometer.
[4] Unicam SP1800 UV spectrophotometer.
[5] Varian A-60 or EM-360 60-MHz spectrometer.
[6] AEI MS-12 or MS-50 mass spectrometer.

H, 8.80; N, 6.32.

2-Dimethylamino-5-oxohexanoic Acid Ethyl Ester (XV)—Compound XIV (2.17 g, 10 mmoles) in 95% ethanol (60 ml) was hydrogenated at 40 psi over 10% palladium-on-charcoal with an excess of formaldehyde (37% solution) for 5 hr. The catalyst was filtered, and the solution was evaporated under reduced pressure. The residue was hydrolyzed with 10% HCl (12 ml) for 6 hr at room temperature. The resulting solution was made alkaline with dilute potassium hydroxide and extracted three times with hot chloroform. The combined extracts were washed once with water and dried. Evaporation under reduced pressure gave 1.5 g (75% overall yield) of the desired compound in a fairly pure state as evidenced by TLC [methanol–chloroform (1:9), silica gel]; IR (neat): 2950, 2930, 2860, 2820, 2780, 1730–1710 (strong, keto and ester carbonyl groups), 1450, 1360, 1170, 1160, and 1030 cm^{-1}; NMR ($CDCl_3$): δ 1.28 (t, 3H, J = 7 Hz, OCH_2CH_3), 2.13 (s, 3H, $COCH_3$), 2.30 [s, 6H, $N(CH_3)_2$], 3.10 [t, 1H, J = 7 Hz, $CHN(CH_3)_2$], and 4.15 (q, 2H, J = 7 Hz, OCH_2CH_3).

Anal.—Calc. for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.51. Found: C, 60.10; H, 9.66.

3-Ethoxy-6-dimethylamino-2-cyclohexen-1-one (XVII)—Sodium hydride (288 mg, 50% suspension in oil, 2 equivalents) in benzene (15 ml) was stirred under dry nitrogen and heated to 40°. The dimethylamino keto ester (XV) (603 mg) then was added dropwise. After addition was complete, the mixture was refluxed for 10 hr. The solid was collected by filtration and washed with benzene. It then was dissolved in water, and the solution was acidified with 2 *N* HCl. The resultant solution was evaporated to dryness *in vacuo*. The solid mass was extracted with ethanol, and the solvent was removed by evaporation.

The salt obtained was dissolved in water and made alkaline with dilute sodium bicarbonate solution. The resultant solution was extracted three times with chloroform. Evaporation of the solvent under reduced pressure gave a liquid, which was purified by TLC [chloroform–methanol (3:1), silica gel]; IR (neat): 2980, 2930, 2860, 2820, 2780 (dimethylamino stretching), 1650 (conjugated carbonyl), 1600 (double bond conjugated with carbonyl), 1375, 1235, 1200, 1170, 1035, 1020, 900, 850, 810, and 690 cm^{-1}; NMR ($CDCl_3$): δ 1.33 (t, 3H, J = 7 Hz, OCH_2CH_3), 2.40 [s, 6H, $N(CH_3)_2$], 3.85 (q, 2H, J = 7 Hz, OCH_2CH_3), and 5.28 (s, 1H, olefinic proton); mass spectrum: parent ion m/z 183.1255 (3.1%) (calc. for $C_{10}H_{17}NO_2$: 183.1255), 140 (32.6), 112 (6.0), 84 (11.3), and 71 (100).

2-(α-Benzoylaminobutanoic acid)-2-methyl-1,3-dioxolane Ethyl Ester (XVIII)—To a stirred solution of XIV (17 g, 80 mmoles) in pyridine (20 ml) and benzene (40 ml) was added dropwise benzoyl chloride (11.5 g, 80 mmoles) in benzene (5 ml). The resulting mixture was heated to gentle reflux for 30 min and poured into 200 ml of water. The benzene layer was separated, and the aqueous solution was extracted twice with benzene. The combined benzene extracts were washed with water and with 5% sodium carbonate solution and dried (anhydrous sodium sulfate). The drying agent was removed by filtration, and the benzene was evaporated to a small volume (10 ml).

Hexane (40 ml) was stirred into the mixture, and the benzoyl derivative crystallized out on standing (13 g, 50.7%). Recrystallization from benzene and pentane gave a white solid, mp 87–88°; IR (KBr): 3340 (amide NH), 3060 (aromatic C–H), 2970, 2880, 1740 (ester C=O), 1640 (amide C=O), 1510, 1490, 1370, 1210, 1190, 1060, 950, 860, 720, and 690 cm^{-1}; NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 6.5 Hz, OCH_2CH_3), 3.92 (s, 4H, OCH_2CH_2O), 4.22 (q, 2H, J = 7 Hz, OCH_2CH_3), 4.75 (t, 1H, J = 6.5 Hz, CH), and 7.0–8.0 (m, 6H, C_6H_5 and NHCO).

Anal.—Calc. for $C_{17}H_{23}NO_5$: C, 63.53; H, 7.21; N, 4.36. Found: C, 63.35; H, 7.27; N, 4.38.

2-Benzoylamino-5-oxohexanoic Acid Ethyl Ester (XIX)—Compound XIX was obtained in quantitative yield from XVIII by hydrolysis in dilute hydrochloric acid at room temperature for several hours; NMR ($CDCl_3$): δ 1.27 (t, 3H, J = 7 Hz, OCH_2CH_3), 2.12 (s, 3H, $COCH_3$), 4.20 (q, 2H, J = 7 Hz, OCH_2CH_3), 4.76 (t, 1H, J = 6.5 Hz, CH), and 7.0–8.0 (m, 6H, C_6H_5 and NHCO).

***N*-(3-Hydroxy-1-oxo-2-cyclohexen-4-yl)benzamide (XX)**—To a stirred mixture of sodium hydride (2.4 g, 50% suspension in oil, 50 mmoles) in dry benzene (150 ml) under nitrogen was added dropwise a solution of XIX (7 g, 25 mmoles) in benzene (20 ml). The mixture was kept at 50–60° for 1 hr and brought to a gentle reflux for 5 hr. The solvent was filtered, and the solid obtained was dissolved in water (150 ml) and acidified with dilute hydrochloric acid. The solution was filtered, and the precipitate was collected. The filtrate was extracted twice with acetonitrile, dried, and evaporated *in vacuo*. The solid obtained was combined with that obtained from filtration.

Recrystallization from acetone gave a white solid, mp 175–177° dec. [lit. (3) mp 175–177°]; IR (KBr): 3300 (amide NH), 3060 (aromatic CH), 2920, 2880, 2700–2500 (broad, associated OH), 1630–1590, 1540–1520, 1340, 1330, 1260, 1200, 920, 840, 710, and 700 cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 4.65 [t, 1H, J = 7 Hz, $CH(NHCOC_6H_5)$], 5.3 [s, 1H, CH=C(OH)], 7.00–8.00 (m, 6H, C_6H_5 and NHCO), and 8.36 [m, 1H, CH=C(O*H*)]; mass spectrum: m/z 231 (16.7%), 203 (98), 185 (7.6), 175 (3.5), 122 (95), 105 (100), and 77 (99); metastable ions were at 178.5, 150.9, 168.5, and 73.3.

Anal.—Calc. for $C_{13}H_{13}NO_3$: C, 67.53; H, 5.66; N, 6.05. Found: C, 67.58; H, 5.79; N, 5.80.

***N*-(3-Ethoxy-1-oxo-2-cyclohexen-6-yl)benzamide (XXI)**—Compound XX (378 mg, 1.6 mmoles) and *N,N,N',N'*-tetramethyl-1,8-naphthalenediamine[7] (428 mg, 2 mmoles) were stirred in anhydrous methylene chloride (10 ml) under a dry nitrogen atmosphere. To the clear solution was added triethyloxonium tetrafluoroborate (1.52 g, 8 mmoles) in methylene chloride (10 ml). After 4 hr of stirring, 20 ml of anhydrous methanol was added, and stirring was continued for 10 hr more. The solvent was evaporated *in vacuo*, and the brown oil obtained was dissolved in ethyl acetate and stirred with an aqueous solution of sodium acetate for 3 hr. The organic layer was separated, and the aqueous portion was extracted twice with ethyl acetate. The combined organic extracts were washed with brine.

Evaporation of the solvent *in vacuo* gave a viscous brown oil (165 mg, 39.8%); IR (neat): 3300 (broad), 3060, 2970, 2930, 1715, 1640 (broad, intense), 1600 (intense), 1575, 1540, 1530, 1485, 1380, 1330, 1230, 1200, 1100, 1035, 980, 920, 710, and 690 cm^{-1}; NMR ($CDCl_3$): δ 1.20 (t, 3H, J = 7 Hz, OCH_2CH_3), 3.50 (q, 2H, J = 7 Hz, OCH_2CH_3), 4.50 [m, 1H, $CH(NHCOC_6H_5)$], 5.45 [s, 1H, CH=C(OC_2H_5)], and 7.00–8.00 (m, 6H, C_6H_5 and NHCO); mass spectrum: parent ion m/z 259.1199 (2.1%) (calc. for $C_{15}H_{17}NO_3$: 259.1204), 245 (2.1), 203 (3.2), 154 (3.7), 138 (19.8), 112 (3.2), 106 (6.5), 105 (100), 98 (20.6), 97 (3.6), 84 (2.5), 77 (72.7), and 68 (34.6).

α-Chloroacetyl Isocyanate—This compound was prepared by the method of Speziale and Smith (15) and was further characterized as its ethyl urethan, $ClCH_2CONHCO_2C_2H_5$, mp 127.5–128°.

Anal.—Calc. for $C_5H_8ClNO_3$: C, 36.27; H, 4.87; N, 8.46. Found: C, 36.18; H, 4.80; N, 8.31.

2-(4-Benzoylamino-3-hydroxy-1-oxo-2-cyclohexen-2-yl)-4(5*H*)-oxazolone (XXII)—Compound XX (462 mg, 2 mmoles) was stirred for 1 hr with a 2 *N* ethanolic solution of sodium ethoxide (1 ml, 2 mmoles). The ethanol was evaporated *in vacuo*, and the resulting sodium enolate of XX was dried *in vacuo* overnight. To the dried sodium enolate in acetonitrile (15 ml) under a dry nitrogen atmosphere was added α-chloroacetyl isocyanate (480 mg, 4 mmoles) from a syringe. The mixture was stirred for 1 hr at room temperature and then refluxed for 8 hr. Water (5 ml) was added, and the mixture was acidified to pH 2 with dilute hydrochloric acid and extracted with ethyl acetate–acetonitrile (1:2). The combined extracts were washed with brine, dried (anhydrous sodium sulfate), and evaporated *in vacuo*.

After evaporation, the brown liquid residue was triturated with chloroform to give 305 mg (48.5%) of XXII; IR (KBr): 3300 (medium), 3060, 2960, 2900, 1790 (intense), 1680 (intense), 1625 (broad, intense), 1600 (strong), 1580, 1520 (broad, intense), 1440, 1400, 1350, 1305 (strong), 1205, 1100, 1060, 1045, 985, 960, 940, 860, 815, 800, 765, 715, and 680 cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 5.00 (s, 2H, CH_2), 7.00–8.00 (m, 6H, C_6H_5 and NHCO), and 8.60 (m, 1H, OH); mass spectrum: parent ion m/z 314.0908 (13.1%) (calc. for $C_{16}H_{14}N_2O_5$: 314.0902), 315 (M + 1) (2.7), 209 (15.8), 194 (5.3), 193 (53.4), 181 (8.9), 167 (5.3), 161 (5.1), 154 (3.2), 151 (4.9), 149 (10.7), 126 (10.4), 125 (3.5), 123 (0.8), 122 (5.0), 106 (8.2), 105 (100), 84 (5.3), 77 (47.8), 68 (5.8), and 67 (4.5).

2-(3-Hydroxy-5,5-dimethyl-1-oxo-2-cyclohexen-2-yl)-4(5*H*)-oxazolone (XXIV)—A solution of dimedone sodium enolate (XXIII) (1.4 g, 10 mmoles) and α-chloroacetyl isocyanate (2.4 g, 20 mmoles) in acetonitrile (30 ml) was refluxed for 6 hr. The solution was acidified with acetic acid (1 ml) and evaporated *in vacuo*. The residue was stirred with water (10 ml) and acidified with dilute hydrochloric acid until distinctly acidic. The acid solution was extracted with ethyl acetate (2 × 40 ml), washed with saturated sodium chloride solution (30 ml), dried, filtered, and evaporated under reduced pressure. The residue was triturated with petroleum ether to give a solid precipitate (1.6 g, 73%).

Recrystallization from acetone afforded a yellow, puff-like solid, mp 227–229°; UV: λ_{max} (ethanol) 236 (log ϵ 3.95) and 290 (4.14) nm; λ_{max} (ethanol–sodium hydroxide) 258 (4.04) and 308 (4.22) nm; IR (KBr): 3140, 2950, 1770, 1670, 1600, 1515, 1430, 1320, 1200, 1060, 980, 820, 750, 680, and 620 cm^{-1}; NMR ($CDCl_3$): δ 1.08 (s, 6H, *gem*-dimethyl groups), 2.48 (d, 4H, J = 8 Hz, methylene groups), 4.85 (s, 2H, CH_2), and 12.5 (s, 1H,

[7] Proton-Sponge.

OH); mass spectrum: parent ion *m/z* 223.0849 (63.7%) (calc. for $C_{11}H_{13}NO_4$: 223.0843), 224 (M + 1) (9.8), 195 (6.4), 167 (33.5), 139 (7.4), 126 (45.0), 111 (100), 83 (61.8), and 67 (23.0).

3-Amino-5,5-dimethyl-1-oxo-2-cyclohexene - 2 - carboxamide (XXV)—Compound XXIV (223 mg, 1 mmole) was dissolved in 5 ml of saturated ammoniacal methanol. After standing at room temperature for 24 hr, the solvent was evaporated *in vacuo*. The residue was dissolved in acetone, from which colorless crystals appeared (115 mg, 63%), mp 188–189° dec.; UV: λ_{max} (ethanol) 260 (log ϵ 64.6) nm; IR (KBr): 3400, 3300, 3200, 3140, 2950, 2930, 2860, 1630 (intense), 1600–1580 (broad, intense), 1470, 1390, 1385, 1380, 1370, 1330, 1280, 1080, 760, and 635 cm^{-1}; NMR (CD_3COCD_3): δ 1.04 (s, 6H, *gem*-dimethyl groups), 2.40 (s, 4H, methylene groups), 3.07 (s, 2H, NH_2), 6.77 (broad s, 1H, CONH), and 10.57 (broad s, 1H, CONH); mass spectrum: parent ion *m/z* 182.1049 (100%) (calc. for $C_9H_{14}N_2O_2$: 182.1054), 183 (M + 1) (10.4), 167 (8.3), 154 (17.4), 139 (6.3), 126 (32.0), 113 (13.2), 98 (17.6), 85 (93.9), 84 (71.7), 71 (8.4), 70 (27.5), 68 (26.0), 57 (10.4), 56 (17.2), and 55 (21.7).

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Determination of Platinum in Serum and Ultrafiltrate by Flameless Atomic Absorption Spectrophotometry

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Abstract □ A graphite furnace atomic absorption spectrophotometric assay, capable of accurately determining nanogram amounts of platinum in serum and ultrafiltrate, was developed. A sample serum or ultrafiltrate was acidified with nitric acid and heated to destroy the protein–platinum bond. A measured excess of ammonium 1-pyrrolidinedithiocarbamate was added, and the platinum complex was extracted into isopropylacetone. The extract was injected into the graphite furnace. The sample was dried, charred, and atomized using optimal conditions. The resulting absorbance was used to determine the platinum content.

Keyphrases □ Platinum—determination in serum and ultrafiltrate, flameless atomic absorption spectrophotometry □ Atomic absorption spectrophotometry, flameless—determination of platinum in serum and ultrafiltrate □ Analytical techniques—determination of platinum in serum and ultrafiltrate by flameless atomic absorption spectrophotometry

A procedure to determine platinum levels in serums and ultrafiltrates of patients receiving *cis*-diamminedichloroplatinum(II)[1] [$PtCl_2(NH_3)_2$] was investigated. Platinum levels were monitored during a course of treatment using both serum and ultrafiltrate samples.

[1] Platinol.

BACKGROUND

Several analytical techniques to determine platinum in biological samples have been reported, such as neutron activation analysis (1), X-ray fluorescence (2), radioisotope dilution (3), flameless atomic absorption spectrophotometry (4–7), and high-performance liquid chromatography (8). Atomic absorption spectrophotometry has facilitated the determination of minute concentrations of metals in biological fluids. Flameless atomic absorption spectrophotometry was chosen for this study.

Previous procedures for platinum estimation in biological fluids using this technique involved wet-ashing with nitric acid–perchloric acid for sample preparation prior to injection into the graphite furnace. In the present study, two parameters had to be considered, the limited volume of each serum sample and ultrafiltrate and the number of samples to be assayed for platinum content. Wet-ashing was not suitable for the present study. Attempts to determine platinum levels in serum and ultrafiltrate by direct injection into the graphite furnace with no prior sample treatment were unsuccessful. The results obtained by direct injection were highly variable due to sample splatter within the graphite furnace and onto the quartz end-windows during charring.

The present report describes a technique that involves the formation

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