palynological report of the Barakar Formation with coal occurrences in this area. Thus, recognition of Barakar Formation based upon field study is now corroborated by palynological study. Detailed investigation of the spore and pollen complex of the coal seam is under way.

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ACKNOWLEDGEMENT. We thank Sri D. C. Das and Dr K. S. Murthy for encouragement.

30 October 1989; received 1 February 1990

Anticancer drugs part III: New spin labelled derivatives of podophyllotoxin

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Three new spin labelled derivatives of podophyllotoxin, *N'*-deoxypodophyllic acid-*N*-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)] thiosemicarbazide(GP-5,4), *N'*-picropodophyllic acid-*N*-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)] thiosemicarbazide(GP-6,7) and deoxypodophyllic acid-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)] hydrazone(GP-8,8) have been synthesized by the reaction of appropriate nitroxyls with hydrazides of podophyllotoxin.

PODOPHYLLOTOXIN (1) and a number of its derivatives possess anticancer activity and some of them have been extensively used clinically[1,2]. Recently we have found[3–6] that the introducing a stable nitroxyl moiety into the molecule of podophyllotoxin could result in compounds with pharmacological properties superior to those of the parent compounds. It was considered worthwhile to synthesize more spin labelled derivatives of podophyllotoxin to find out a new anticancer drug with high activity and low toxicity. Therefore, in the present work we have synthesized *N'*-deoxypodophyllic acid-*N*-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)] thiosemicarbazide (GP-5,4), *N'*-picropodophyllic acid-*N*-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)] thiosemicarbazide(GP-6,7) and deoxypodophyllic acid-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)] hydrazone(GP-8,8).

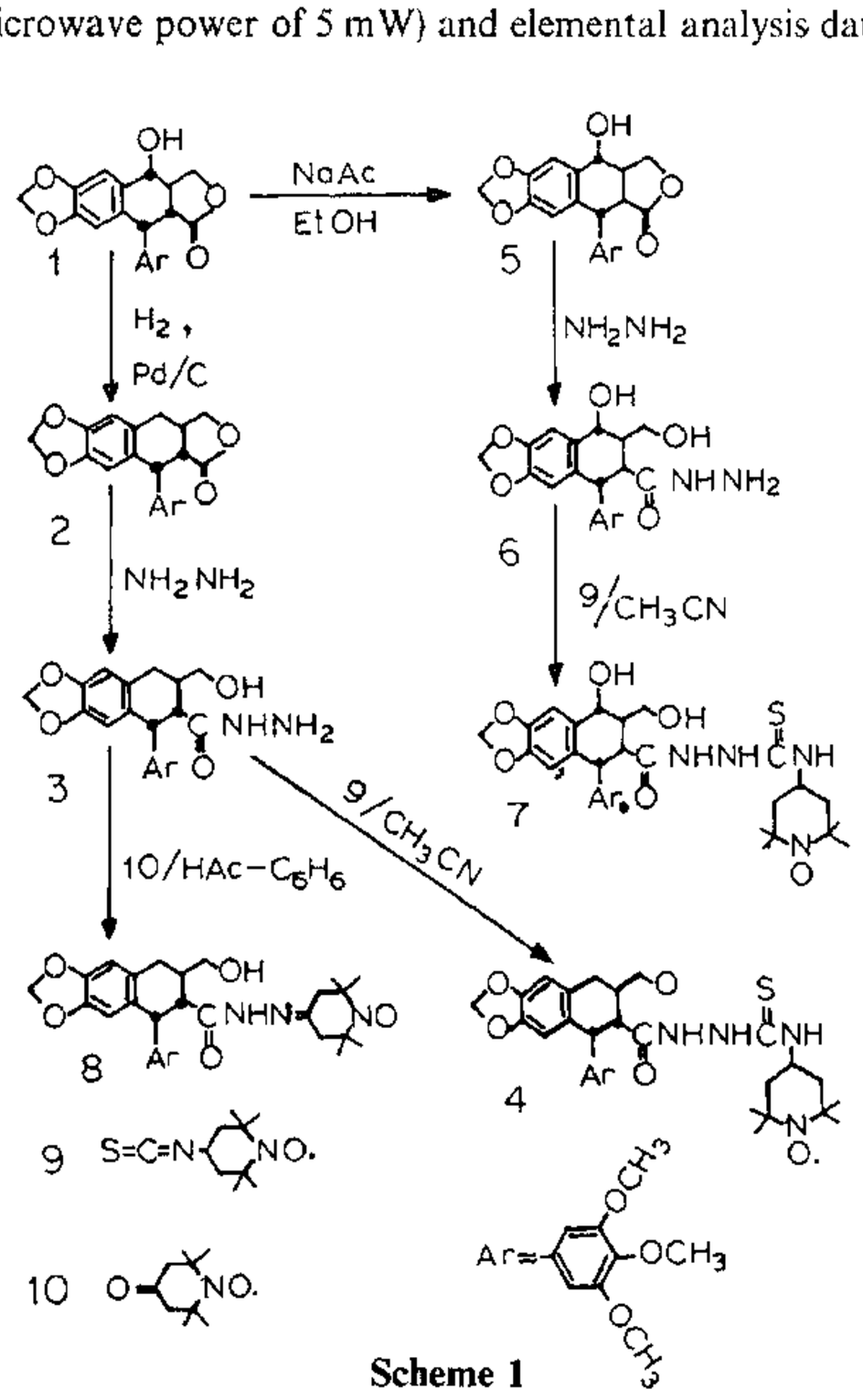
N'-deoxypodophyllic acid-*N*-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)] thiosemicarbazide(4), *N'*-picropodophyllic acid-*N*-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)] thiosemicarbazide(7) and deoxypodophyllic acid-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)] hydrazone(8) were prepared as shown in Scheme 1.

Podophyllotoxin(1), isolated from a Chinese medicinal herb *Podophyllum emodi* Wall var. *Chinesis sprague*, was used as the starting material.

Podophyllotoxin(1) was converted to deoxypodophyllotoxin(2) by Pd/C catalytic reduction. Hydrazinolysis of 2 yielded deoxypodophyllic acid hydrazide(3). GP-5(4) was prepared by the reaction of 3 with isothiocyanide(9). The treatment of 3 with excess 2,2,6,6-tetramethyl-1-piperidinyloxy(10) gave GP-8(8). The picropodophyllic acid hydrazide(6) (ref. 7) obtained from the hydrazinolysis of picropodophyllotoxin(5) was converted to GP-6(7) under similar conditions.

All products were purified by chromatography on silica gel and checked by thin layer chromatography with 3–5 different solvent systems. Structural characterization is based on elemental analysis, MS, IR, UV and ESR data.

IR spectra were recorded on 5DX infrared spectrophotometer (ν_{max}^{KBr} cm^{-1}) and UV spectra on Beckman DU-7 ultraviolet visible spectrophotometer (λ_{max}^{MeOH} nm). MS data were obtained with a ZAB-HS mass spectrometer (important fragments are given with the relative intensities in the bracket), EPR data with Bruker ER-200D-SRC electron paramagnetic resonance spectrometer (X band, modulation frequency of 12.5 kHz, microwave power of 5 mW) and elemental analysis data



Scheme 1

with 1106 elementary autoanalyser. Melting points are uncorrected.

Deoxypodophyllotoxin(2)

A mixture of 10% Pd/C catalyst (1.8 g), glacialacetic acid (45 ml) and podophyllotoxin(1) (2 g, 4.8 mmol) was reacted with hydrogen at 95°C for 10 h. The reaction mixture was filtered and the catalyst was washed with hot glacialacetic acid. The filtrate was completely evaporated under vacuum. The residue was recrystallized from methanol, yield 1.54 g (88%), m.p. 166–168°C; MS (EI): *m/z* 398(M^+, 100), 383(10), 353(8), 339(12), 185(22), 181(34), 173(28), 168(17); IR: 1775(C=O), 1590, 1500 and 1480 (Ar)cm^{-1}.

Deoxypodophyllic acid hydrazide(3)

A mixture of glacialacetic acid (0.12 ml), anhydrous hydrazine (0.12 ml), methanol (0.6 ml) and 2(0.6 g, 1.5 mmol) was refluxed for 1 h on water bath. The reaction mixture was completely evaporated under vacuum. The residue was dissolved in hot water and filtered. Hexane was added dropwise to the filtrate until no more colourless precipitate formed, yield 0.28 g (42%), m.p. 150–155°C. MS (EI): *m/z* 430(m^+, 100), 412(10), 399(36), 398(74), 381(24), 353(28), 339(40), 198(98), 181(64); IR: 3640–3360(OH, NH), 1680(C=O), 1630, 1590 and 1480(Ar)cm^{-1}.

GP-5(4)

A solution of 3(0.10 g, 0.23 mmol) and isothiocyanide(9) (0.05 g, 0.23 mmol) in dry methylcyanide (5 ml) was refluxed for 1 h. The reaction mixture was completely evaporated under vacuum. The crude product was chromatographed through silica gel using CH_2Cl_2–acetone (2:1, v/v) as eluent, yield 0.065 g (44%), m.p. 188–190°C. MS (FAB): *m/z* 645(M^++2, 12), 644(M^++1, 30), 643(M^+, 40); IR: 3500–3300(OH,NH), 1694 (C=O), 1588, 1504, 1483(Ar), 1365(N–O), 1230 (C=S) cm^{-1}; UV: 291(3950), 243(16120), 216(18230) nm; EPR: g_o = 2.0058, $\Delta Ho = 2.61$ Gs, $A_N = 16.10$ Gs (triplet peak, in 1×10^{-4} M EtOH); Anal: Found C, 59.71; H, 7.00; N, 8.63% (Calcd. for $C_{32}H_{43}N_4O_8S$, C, 59.70; H, 6.75; N, 8.70%).

GP-6(7)

A solution of picropodophyllic acid hydrazide(6) (2.23 g, 5 mmol) and isothiocyanide(9) (1.07 g, 5 mmol) in dry methylcyanide (100 ml) was refluxed for 2 h. Removal of methylcyanide under reduced pressure afforded an orange-yellow solid. The crude product was chromatographed through silica gel using CH_2Cl_2–acetone (1:1, v/v) as eluent, yield 1.68 g (51%), m.p. 183–185°C. MS (FAB): *m/z* 661(M^++2,9), 659(M^+,3), 643(5); MS(EI): *m/z* 414(84), 396(22), 183(30), 124(100). IR: 3500–3300 (OH, NH), 1694(C=O), 1588, 1510, 1483(Ar), 1365(N–O), 1236(C=S) cm^{-1}; UV: 291(3350), 244(14730) and 218 (15140) nm; EPR: $g_o = 2.0061$, $\Delta Ho = 2.66$Gs, A_N = 16.19Gs (triplet peak, in 1×10^{-4} M EtOH). Anal: Found C, 58.19; H, 6.56; N, 8.40% (Calcd. for $C_{32}H_{43}O_9N_4S$, C, 58.27; H, 6.58; N, 8.50%).

GP-8(8)

A mixture of deoxypodophyllic acid hydrazide(3) (0.60 g, 1.4 mmol), 4-oxo-2,2,-6,6-tetramethyl-1-piperidinyloxy(10) (0.51 g, 3 mmol) and dry benzene (10 ml) in the presence of glacialacetic acid (4 drops) as catalyst was refluxed for 2 h. After removal of solvent, the crude product was chromatographed twice through silica gel using CH_2Cl_2–MeOH (12:1, v/v) as eluent and CH_2Cl_2–acetone (1:1, v/v) as eluent. A orange-yellow powder was obtained, yield 0.41 g (50%), m.p. 118–120°C, $[\alpha]_D^{20} = -212°$ (C=0.43, EtOH). MS(EI): *m/z* 583 (M^++1,12), 582(M^+,2), 568(10), 567(14), 510(20), 398(100). IR: 3500–3400 (OH,NH), 1750(C=O), 1580, 1500(Ar), 1365 (N–O) cm^{-1}. UV: 295(5530), 228(24600), 207(55000) nm EPR: $g_o = 2.0057$, $Ho = 1.70$ Gs, $A_N = 15.23$ Gs (triplet peak, in 1×10^{-4} M EtOH). Anal: Found C, 63.88; H, 7.14; N, 7.05% (Calcd. for $C_{31}H_{40}O_8N_3$, C, 63.92; H, 6.88; N, 7.20%).

The above compounds were tested for antimitotic activity by following the method described earlier[8] and was found to be less active ($ID_{50} = 4.6 \times 10^{-5}$ for 8 and $ID_{50} = 1.9 \times 10^{-5}$ for 4 respectively) compared to the parent compound 2($ID_{50} = 8.0 \times 10^{-8}$ M). Compound 7 is inactive for antimitotic activity.

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ACKNOWLEDGEMENT. The financial support of the Fund of Applied Organic Chemistry Laboratory, Lanzhou University, is gratefully acknowledged.

14 August 1989